This document was created in response to a Freedom of Information request made to CSIRO.

FOI Number:          FOI2011/69
Date:                31 July 2012
Request:             Documents relating to the outbreak of a herpes-like virus in Victoria abalone, now known as Abalone Viral Ganglioneuritis ("AVG") which commenced during December 2005 (the "Victorian Outbreak")
Documents:           Part 4 – Document 77

For more information, please refer to CSIRO’s FOI disclosure log at www.csiro.au/FOILog
Good Morning Mark

Nice to meet you yesterday.

Attached are the presentations.

Enjoy your weekend.

Kind Regards

Caroline McGowan
EA to Dr Mehdi Doroudi DVM, PhD
Research Director
Marine and Freshwater Systems
PIRVic
Department of Primary Industries

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Email: caroline.mcgowan@dpi.vic.gov.au

(See attached file: FINAL Abalone Virus Scientific Forum - Peter Appleford Introduction presentation 21 Sept.pdf)
(See attached file: FINAL Abalone Virus Scientific Forum - Research Priorities 21 Sept.pdf)
(See attached file: FINAL Abalone Virus Scientific Forum Hugh Millars presentation 21 Sept.pdf)
(See attached file: FINAL Abalone Virus Scientific Forum Judith Handlinger - Abalone survey & surveillance need.pdf)
(See attached file: FINAL Abalone Virus Scientific forum Mark Crane presentation 21 Sept.pdf)
(See attached file: FINAL Abalone Virus Scientific forum Mehdi Doroudi presentation 21 Sept.pdf)
(See attached file: FINAL Abalone Virus Scientific Forum Paul Hardy-Smith presentation 21 Sept.pdf)
(See attached file: FINAL Abalone Virus Scientific Forum Peter Appleford - Way Forward.pdf)
(See attached file: FINAL Abalone Virus Scientific Forum Tristan Renault-Herpesvirus-Australia-2006.pdf)
Hi Caroline,

Apologies for bothering you so early after the meeting yesterday. I have a FRDC Aquatic Animal Health Subprogram (AAHS) meeting next week and the committee members will be very interested in receiving a report about the various abalone-related activities this week. You did mention that we would get copies of all the presentations made yesterday - if I could get these sooner rather than later it would greatly assist me in putting together a summary report for the AAHS.

Appreciate your assistance.

Mark

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Australian Animal Health Laboratory
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e-mail: mark.crane@csiro.au

-----Original Message-----
From: Caroline.McGowan@dpi.vic.gov.au
[mailto:Caroline.McGowan@dpi.vic.gov.au]
Sent: Tuesday, 19 September 2006 17:15
To: Crane, Mark (LI, Geelong)
Subject: RE: Powerpoint presentation

Great - see you on Thursday

Caroline McGowan
EA to Dr Mehdi Doroudi DVM, Phd
Research Director
Marine and Freshwater Systems
PIRVic
Department of Primary Industries
No problems, Caroline

Cheers

Mark

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-----Original Message-----
From: Caroline.McGowan@dpi.vic.gov.au
[mailto:Caroline.McGowan@dpi.vic.gov.au]
Sent: Tuesday, 19 September 2006 16:54
To: Crane, Mark (LI, Geelong)
Subject: Re: Powerpoint presentation

Mark

I've made a couple of format changes (bullet points etc - hope that is ok).
I've saved it as a pdf version should you want to forward it electronically
to anyone.

Please let me know if the attached is ok?

Kind Regards
Hi Caroline,

You should have rec'd 2 emails with my presentation in 2 parts; part 1 (labelled ...part 11) is the first half and part 2 is the remainder. If you could let me know that you have rec'd these OK and you can put these together that would be great.

Cheers

Mark

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AGENDA

09.30 Registration

10.00 Welcome, Introduction & Background
   Chair - Dr Peter Appleford, Executive Director Fisheries Victoria

10.30 DPI's Response
   Dr Hugh Miliar, Chief Veterinary Officer, Biosecurity Victoria

11.00 Epidemiological aspects
   Dr Paul Hardy-Smith, Managing Director, Panaquatic Health Solutions Pty Ltd

11.30 Open Forum with Panel Experts
   Dr Peter Appleford - Chair
   Dr Carolyn Friedman, United States of America
   Dr Judith Handlinger, Tasmania
   Dr Mike Hine, New Zealand
   Dr Anna Mouton, South Africa
   Dr Tristan Renault, France

12.30 Lunch

13.15 Research undertaken to date
   Dr Mark Crane, Project Leader, AAHL Fish Diseases Laboratory, CSIRO

13.45 Current research proposals
   Dr Mehdz Doroudi, Research Director, Marine & Freshwater Systems, PRVic

14.15 Future directions
   Dr Peter Appleford, Executive Director Fisheries Victoria

14.30 Open Forum with Panel Experts
   Dr Peter Appleford - Chair
   Dr Carolyn Friedman, United States of America
   Dr Judith Handlinger, Tasmania
   Dr Mike Hine, New Zealand
   Dr Anna Mouton, South Africa

15.00 Close
Value of the Abalone industry in Victoria

Abalone Commercial 1.490 T $60 m
Aquaculture 124 T $4.4 m
Previous reported diseases of abalone in Australia and Victoria

Pathogens and parasites recorded from wild stock

<table>
<thead>
<tr>
<th>Findings</th>
<th>TAS</th>
<th>NSW</th>
<th>WA</th>
<th>SA</th>
<th>VIC</th>
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<td>Negative</td>
<td>Positive</td>
<td>1 positive</td>
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<td></td>
<td></td>
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<td>1 Negative</td>
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<td>NA</td>
<td>1 Rare</td>
<td>NA</td>
<td>NA</td>
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<td></td>
<td>1 Rare</td>
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<tr>
<td>Bacterial infection</td>
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<tr>
<td>5 types</td>
<td>3 Rare</td>
<td>NA</td>
<td>2 Rare</td>
<td>1 Rare</td>
<td>NA</td>
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<tr>
<td>Fungal infection</td>
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<td>Post-survey of uncertain origin</td>
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<tr>
<td></td>
<td>0 known to occur</td>
<td>0 known to occur</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Protozoa &amp; protozoan like parasites</td>
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<tr>
<td>13 Types</td>
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<td>1 Common</td>
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<td>1 Common</td>
<td>1 Common</td>
<td>5 Rare</td>
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<td>2 Moderate</td>
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<td>1 Rare</td>
<td>1 Variable</td>
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<td>7 Rare</td>
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<td>1 Restricted</td>
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<td></td>
<td>1 – 0 known to occur</td>
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<tr>
<td>Metazoan parasites</td>
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<tr>
<td>3 types</td>
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<tr>
<td></td>
<td>1 Rare</td>
<td>NA</td>
<td>2 Common</td>
<td>1 Rare</td>
<td>2 Rare</td>
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<tr>
<td></td>
<td>0 known</td>
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<td></td>
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<td></td>
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</table>
Pathogens and parasites recorded from farmed stock

<table>
<thead>
<tr>
<th>Findings</th>
<th>TAS</th>
<th>NSW</th>
<th>WA</th>
<th>SA</th>
<th>VIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Perkinus</strong></td>
<td>2 Negative</td>
<td>1 Negative</td>
<td>2 Negative</td>
<td>2 Negative</td>
<td>2 Negative</td>
</tr>
<tr>
<td><strong>Inclusion-like bodies</strong></td>
<td>2 types</td>
<td>0</td>
<td>NA</td>
<td>1 Rare</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td>5 types</td>
<td>2 Rare</td>
<td>NA</td>
<td>1 Past infection suspected</td>
<td>1 Rare</td>
</tr>
<tr>
<td><strong>Fungal infection</strong></td>
<td>1 type</td>
<td>1 Rare</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Protozoa &amp; like parasited</strong></td>
<td>13 types</td>
<td>6 Rare</td>
<td>1 Rare</td>
<td>3 Rare</td>
<td>3 Rare</td>
</tr>
<tr>
<td><strong>Metazoan parasites</strong></td>
<td>3 Types</td>
<td>1 Rare</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>
The extent of infection in wild abalone population
Sampled 31st January 2006

- Virus found at site
- Virus not found at site

0 Days (1st sampling day)
Sampled 24th May 2006

- Virus found at site
- Virus not found at site

113 Days
Sampled 25\textsuperscript{th} May 2006

- Virus found at site
- Virus not found at site

114 Days
Sampled 25th May 2006

- Virus found at site
- Virus not found at site

114 Days
Sampled 31st May 2006

- Virus found at site
- Virus not found at site

120 Days
Sampled 2nd June 2006

122 Days

- Virus found at site
- Virus not found at site
Sampled 20th June 2006

140 Days

- Virus found at site
- Virus not found at site
Sampled 25th July 2006

- Virus found at site
- Virus not found at site
- Virus gone from site

175 Days
Sampled 26th July 2006

- Virus found at site
- Virus not found at site
- Virus gone from site

176 Days
Sampled 27th July 2006

177 Days

- Virus found at site
- Virus not found at site
- Virus gone from site
Sampled 7th August 2006

- Virus found at site
- Virus not found at site
- Virus gone from site

186 Days
Joint DPI/Industry Abalone Disease Reference Group

- A joint DPI / Industry Consultative Committee to respond to the disease outbreak
  - Wild catch
  - Aquaculture
  - Processor
  - Government

- Meet as required to transfer information and consider response options
- Being transformed into “Strategic Abalone Biosecurity Working Group”
DPI Response Structure

Reference Group

Incident Controller

Biosecurity Victoria
CVO
Fisheries Victoria

Surveillance Management

Field Response Management

Legislation Instruments

Research Advisory Group

Contractor

Biosecurity

Compliance

Science
Technical and management issues that have been under investigation since January 2006

- Histopathology
  - Gribbles
  - DPI
  - Judith Handlinger
  - International Experts

- Epidemiology
  - Consultants
  - DPI
  - International Experts

- Virology
  - DPI
  - AAHL
  - International Experts

- Biosecurity
  - DPI
  - FRDC
  - International Experts

- Stock Assessments
  - DPI International
Introducing the Expert Panel:

- Dr Carolyn Friedman, USA
- Dr Judith Handlinger, Tasmania
- Dr Mike Hine, New Zealand
- Dr Anna Mouton, South Africa
- Dr Tristan Renault, France
Acknowledgments

- DPI - FV, BV, PIRVic, CAS
- Industry - SIV, WADA, VADA, VAGA
- FRDC
- AAHL
- Panaquatic
- Gribbles
- Contract Divers
Research Priorities

To Do Now:
• Develop & Implement Australia-wide sampling regimes – make bench mark collections, and long term system for collecting apparently diseased samples

A: Diagnostic Tools
• Initially Use Available non-specific tools
• Develop Specific Tools
  – Purification of Virus
  – PCR – polymerase chain reaction
  – Comparison with other Herpes and like viruses

B: Patterns of Disease (EPIDEMIOLOGY)
• Distribution of virus / disease in abalone
• Host range (other species – especially gastropods?)
• Agent Characteristics; (Transmission, Latency, Impact on Larvae, Stability, Disinfection)
Ganglioneuritis in abalone - current status in Victoria

Dr Hugh Millar, CVO
21st of September 2006
Introduction

- Clinical signs
- The agent
- Spread of disease
- Source
- Control measures
- Challenges
- The future
Aims of the Project

- To support disease zoning programs
- Develop on-going health surveillance
- Find out what diseases we have (and where)
- Develop and expand diagnostic expertise
- and trade access movements within Australia.
In 5 States

In conjunction with laboratories 
Judith Handlienger, TAFI / DPIWE

Principal Investigator:

Programs

Development of health surveillance
Translocation issues & the
Speciation - to support trade and
Commercially exploited abalone

A national survey of diseases of

Australian Government
Animal health management

Framework for aquatic techniques

Further information on epidemiology and potential control

Diagnostic tests

Biosecurity plans

Research and development

Improve biosecurity in all sectors of the abalone industry

Integrated fisheries and disease management

The future
Challenges

- Possible endemic (sub-clinical) disease in wild abalone
- No mechanism for compensation
- Surveillance based on clinical disease only
- No diagnostic test for living, (apparently) healthy eg. Host range, distribution, latency
- Limited knowledge about epidemiology
- Detection in wild population

abalone
Response

Department of Primary Industries

Communications
- This strategic forum
- Media
- Angus, website, FGC

Research
- AGCMed

National Consultation
- Incident Control Team established
- Compliance enforcement activities
- Control area declaration

Control measures
- Industry briefing
- Government Australia Disease Reference Group
- Simiar within weeks of dx

Industry engagement
- Stalled, absence, reporting, processors etc
- Begin immediately

Surveillance
- Transmission risks
- Literature review, information, contact - Taiwan and China
- Source spread, disease pattern

Epidemiological Investigation
- Incubity period, on site visits
- On-farm controls and movement restrictions

Immediate Response on Farms
- Epidemiology - electron microscopy
- Rapid diagnosis
Communication - Agnotes, website, FAQs

Encourage diver submission of suspect cases

Surveillance at processing plants

Structured surveillance of wild abalone populations

Surveillance

Incident Control Team established

Replaced by Fisheries Order - in place

Could not control spread via water, animal activity

Aimed to limit spread due to human activity

9th June 2006 - for 60 days

Control Area declared around Port Fairy

Control measures
Sentinel restocking and monitoring program

Culling of all stock with disposal and decontamination

Biocontainment on affected farms

Processing

Only clinically healthy stock moved off for

Affected farms to other farms

Voluntary restrictions on abalone movement from

(9gngliomenuiteis)

Must notify suspicion of herpes-like virus infection

Must notify suspicion of herpes-like virus infection

Act

Notifiable disease (Livestock Disease Control

Rapid diagnosis

Control measures
Source

Exotic source unlikely

Feed source unlikely

Current feed locally produced

Epidemiology not consistent with feed-borne infection

Infected and uninfected tanks same feed

Local spread between contiguous tanks

Possibly introduced with wild stock
Spreading of Disease

- Dilution
- Role of water
- Transmission studies at AAL
  - Reproduced characteristic pathology
  - Waterbath - death at 3 days
  - IM injection - death at 2 days

Taiwanese study:
Indirect contact - Seawater

Direct contact between abalone

Concurrent disease (e.g., Vibrio sp.)

Spawning

Water temperature (>17.5-18 °C)

High stocking density

Role of stress factors

Latent infection - Highly likely

Significant mortalities after 10-14 days

Incubation period 2-14 days

Spread of disease
Herpes virus identified within nerve cells

Inflammation of the nerves (termed 'ganglionitis')

Continued to nervous system pathology

The agent
affected

Suggestion that young stock are more seriously

Affects greenlip, blacklip and hybrid abalone

Ablalone easily removed (or fall off) substrate

Curling of the foot

Protruding radula

Enlarged mouth parts

Mortality within tanks: 5% to 90%

Increased mortality

Clinical signs
Background

At the first National Abalone Convention I indicated that "Australia has some of the world's largest wild harvest abalone industries,

but like most of the world has little data on abalone diseases."

It was recognized that this put at risk good management of wild & aquaculture stocks, and potentially also markets (especially live product)
Disease impacts at that time

- Withering syndrome of Californian abalone had been recognized internationally as a disease of concern. (Now listed as Notifiable to OIE (the international body for disease control).

- Viral diseases was having a major impact in China\(^1\), and soon became officially listed for the Asia-Pacific region as an unknown disease of a serious nature.\(^1\) Goufan Zhand et al (Review, 5th International Abalone Symposium)

- Now proposed that this be fully listed as Notifiable by OIE
- The Australian virus is similar and may fit into this category.
Test methods: Histopathology
### Over 3000 animals examined:  (61 wild sites)

<table>
<thead>
<tr>
<th>State</th>
<th>Wild sites</th>
<th>Farmed sites</th>
<th>No abalone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tas</td>
<td>11 (Blacklip)</td>
<td>9 (Blacklip, &amp; Greenlip)</td>
<td>396 wild Blacklip</td>
<td>912</td>
</tr>
<tr>
<td></td>
<td>1 Greenlip</td>
<td></td>
<td>21 wild Greenlip</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>544 farmed</td>
<td></td>
</tr>
<tr>
<td>Vic</td>
<td>5 Blacklip</td>
<td>5</td>
<td>150 wild blacklip</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>2 Greenlip</td>
<td></td>
<td>60 wild greenlip</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>90 farmed blacklip</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150 farmed greenlip</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>9 sites</td>
<td>9 Greenlip</td>
<td>118 wild Blacklips</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>156 wild greenlip</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>236 farmed greenlip</td>
<td></td>
</tr>
<tr>
<td>WA</td>
<td>18</td>
<td>2 (Greenlip 160)</td>
<td>239 <em>H. roei</em>, 424 <em>H. laevigata</em>, 9 <em>H. conicopora</em> (2 sites), 12 tropical <em>H. asinina</em> (1 site).</td>
<td>687</td>
</tr>
<tr>
<td>NSW</td>
<td>15 Blacklip (Eden to Crowdy Head)</td>
<td>2 (hatchery)</td>
<td>407 Blacklip wild, 99 Blacklip hatchery 1 y.o, 98 <em>H. Coccocoradite</em> spat</td>
<td>604</td>
</tr>
<tr>
<td>Total</td>
<td>(plus some opportunist extras)</td>
<td></td>
<td></td>
<td>3163</td>
</tr>
</tbody>
</table>
Project outline: One round disease survey across Tas, Vic, SA, NSW and WA.
Wild abalone sampling – Victoria

- Wild blacklip sampled Jan & Feb 2003: Discovery Bay, Lady Julia Percy Is, Port Phillip Bay, Cape Schank and Western Port Bay
- Wild greenlip from Walkerville and Portland during the summer of 2004.
- 30 animals / site* (Clusters to test Victoria - not intended to prove freedom at each site – but would have picked up any outbreaks of this disease where a lot of animals are affected)
Findings from Victoria:

The only agents seen are common to all States

- A few parasites not associated with disease
  - Gill ciliates
  - Foot worms
  - Occasional protozoa in oesophagus
- No evidence of *Perkinsus*
- Perkinsus was associated with the severe mortality in wild H. rubra, which appears to have spread from the Terrigal region of NSW coast since 1992
- Animals with *Perkinsus* infection usually showed no gross signs of infection, and appear to die during acute infection, so differ from SA outbreaks
- A few parasites, no other significant findings
Growth Checks and Increased tissue pigment
- Wild Blacklip, Tas, Kent Group
Haemocyte parasite

- Limited distribution, high proportion of animals infected, At low levels very hard to detect).
- Poor growth, heavy pigment (chronic disease), poor stress tolerance.
- Recommended to prevent spread - caution regarding translocation until fully assessed.
Western Australia

- More metazoan (worm) parasites in tissues
- Occasionally damage around the nerves associated with these parasites.
- Other parasites & bacteria similar to other states
- One equivocal *Perkinsus* test in a health abalone that we could not confirm by histology or re-sampling.
- Similar range of parasites
- No nerve inflammation, but one parasite tracking up nerve sheaths
Results summary

- Overall confirmed the diseases we had seen previously - better idea of their distribution
- Several new parasites from wild stocks, but only one associated with poor health
  - From Bass Strait associated with chronic poor growth – consider translocation risk
- Occasional slight chronic reactions round nerves in WA appear to be due to parasites tracking up the nerve sheath
- Some virus-like changes checked out, but no virus found (not ganglioneuritis like)
Survey Conclusions (2005) - critical factors

- This provides a base-line only - health surveillance must continue.
- Health surveillance must cover both aquaculture and the wild industry. (Abalone farmers first request)
- Need for ongoing industry familiarisation with disease - “eyes & ears” to ensure investigation of all disease outbreaks.
- But: these programs still under development and
- Analysis of this virus outbreak identified biosecurity issues (in both sectors) & lack of health surveillance & awareness as key issues.
Need for health surveillance

- It’s your best protection
- There are no doubt other diseases out there
- Need testing to establish disease free zones for trade purposes (live trade)
- As well as targeted testing need an effective early warning system in place.
OIE guidelines for demonstrating disease freedom outside an infected area

- **Need to continue to monitor and test the buffer zone.**
- Over time, could regain status of infected area, if disease eliminated (unlikely)
OIE - pathways to freedom (recognition)

1. Absence of susceptible species
2. Historically free
3. Last occurrence within the previous 25 years
4. Previously unknown disease status

- Meet prescribed biosecurity conditions
- Implement targeted surveillance (Section B)
  - Meet prescribed biosecurity conditions

→ Freedom from Infection

- Maintain prescribed biosecurity conditions
- Discontinue targeted surveillance
OIE - Prescribed biosecurity conditions

- Apply to a particular disease in a particular zone
- *designed to provide assurance that if the disease were present, the Competent Authority would known of it.*
- Need:
  - the disease to be legally notifiable to the Competent Authority;
  - an early detection system is in place
  - infection is not known .. in wild populations
- Import (movement) requirements to prevent the introduction of disease or infection into the zone.
  - Movements from same or better status only.
Early Detection System

- "means an efficient system for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* situation, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority*, with the aim of activating diagnostic investigation with minimal delay".

- **Includes:**
  - Broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in *processing*, of the characteristic signs of the *listed diseases* and *emerging diseases*;
  - Trained veterinarians; competent laboratories; ability to investigate.
Key issues:

- Another key issue identified was the lack of compensation mechanisms for aquatic animals. (Disease control kill-out.)
fin
Abalone Herpesvirus – Results of current research

Mark Crane², Malcolm Lancaster¹ & Serge Corbeil² & Ken McColl²

¹PIRVic Attwood, Department of Primary Industries, Attwood Victoria 3049
²CSIRO Livestock Industries, Geelong Victoria 3220
Abalone herpesvirus: Background

- Histopathological examination on moribund animals indicated a ganglioneuritis

- Examination by electron microscopy revealed the presence of a herpes-like virus in the pleuropedal ganglion

- A similar case was reported in cultured abalone, *Haliotis diversicolor supertaxa*, in Taiwan (Jan 2003) (Chang et al., *Dis Aquat Org* 65: 23-27 2005)
Immediate Research Need

- As discussed with DPI Victoria: Information on the virus transmissibility was required in order to assist implementation of effective management strategies to control the disease and/or eradicate the virus.
- Two infection trials were planned and undertaken.
Objectives:

- Confirm that the virus isolated from sick abalone can cause disease in healthy abalone
- Confirm Henle-Koch’s postulates
- Determine if moribund abalone (virus infected) can transmit the virus to healthy uninfected abalone through the water column
Method:

- Inocula were prepared from 6 frozen (infected) and 9 moribund abalone
- Ganglia were dissected, pooled (1 frozen group and 1 fresh group), homogenised, centrifuged, supernatant filtered and kept at 4°C until inoculation into healthy abalone
Herpes-like virus transmission trial 1

Treatment Groups
(12-15 abalone/group)

1) Co-habitation with sick abalone
2) Injected with frozen virus (100uL)
3) Injected with fresh virus (100uL)
4) Injected with DMEM only (100uL)

Experiments were carried out in 100 L plastic tanks containing 80 L aerated salt water (sea). Water flow rate through the system was 3 Litres/hour
Herpes-like virus transmission trial 1

- An extra tank contained healthy naïve abalone as negative (no treatment) control
- Health status and mortality was recorded daily
- Histological examination and electron microscope analyses were performed on some moribund animals
Herpes-like virus transmission trial 1: Results

Cumulative mortality in abalone

- Co-habitation
- Fresh virus injected
- Frozen virus injected
- DMEM injected
- Untreated

Days post-exposure to virus

% mortality

0 20 40 60 80 100

1 2 3 4 5 6
Conclusions

- The virus is transmitted through the water column from sick abalone to the healthy abalone.
- The virus is highly pathogenic, killing abalone within a few days of infection.
- Injection of the virus in the abalone foot causes disease and mortality.
- The virus remains virulent and pathogenic after being frozen at -80°C.
Objective:

To determine if dilution of contaminated water is a sufficient means of disease control
Method:

- Sample 200L of farm water (on-going abalone mortalities) and transport to AAHL
- Expose healthy abalone to various dilution levels (100%, 10%, 1%, 0.01%, 0.001%) of farm water for a 48 hour period
- Record morbidity and mortality
- Perform histological examination of moribund animals
Results:

- No disease or mortality in any of the experimental groups

- Positive control group (virus-injected abalone) demonstrated typical clinical signs and mortality
Conclusions:

1) There was no (or little) virus present in the water at the time of sampling (virus titres may fluctuate in a farm setting, particularly in flow-through systems) and/or

2) The transportation of the water to AAHL affected the viability of the virus present in the water and/or

3) The virus in the water was present at a titre that was too low to cause a productive infection in the abalone
Herpes-like virus transmission trial 2: Water dilution and virus transmission

Control Experiment:

- Co-habitation of healthy abalone with diseased animals (held in basket) until approx. 50% mortality

- Expose healthy abalone to various dilution levels (100%, 10%, 1%, 0.01%, 0.001%) of simulated farm water from the co-habitation group for a 48 hour period

- Record morbidity and mortality

- Perform histological examination of moribund animals
Herpes-like virus transmission trial 2: Water dilution and virus transmission

Results

Cumulative mortality in abalone exposed to virus infected water

- Co-habitation
- 100% water
- 10% water
- 1% water
- 0.01% water
- 0.001% water
- Untreated control

Days post-exposure

% mortality
Conclusions

The virus remained infectious to animals even after a 1 in 100 dilution (although only one of the duplicate tanks was affected at this dilution compared with both tanks affected at 100% and 10% levels).

Even though the dilution factor at the outlet of the farm is greater than 1 in 100 and while dilution reduces infectious dose, it remains possible that wild mollusc species could become infected by virus released into the environment.
What we know:

- The disease can be transmitted horizontally
- The virus is transmitted through the water column – direct contact is not required for transmission
- The virus is highly pathogenic, killing abalone within a few days of infection
- Injection of the virus in the abalone foot causes disease and mortality
- The virus remains virulent and pathogenic after being frozen at -80°C
- Cannot be grown in fish cell lines
- Oyster herpes virus PCR negative
Abalone Herpes-like virus
Research Needs

What we need to know:

- Geographic range
- Is it the same as the Taiwanese virus?
- Host range
- Distribution and prevalence
- Infectious dose
- Sensitivity to physico-chemical conditions
- Stability in the environment
- Routes of transmission
- Tissue distribution
- Mechanisms of resistance, if any
- Control methods
Abalone Herpes Like Virus current research projects

Mehdi Doroudi DVM, PhD and Harry Gorfine PhD
Research Director, Marine and Freshwater Systems - PIRVic
Development of management strategies for herpes virus infection of abalone

Objectives:

- To establish good biosecurity practices for the abalone wild fisheries and aquaculture

- To develop codes of Practice for the
  - Abalone aquaculture industry
  - Abalone wild harvest industry
  - Abalone processing industry

- To prevent new disease incursions and the control of current disease events virus
Funding and support:

- FRDC
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Methodology:

- Literature & expert reviews
  - Viruses of mollusc (national & international)
  - Biosecure management strategies for mollusc aquaculture & wild commercial fisheries (harvesting & processing)
  - Existing codes-of-practice

- Review existing industry practices
  - On-site activities reviewed
    - Wild harvest, aquaculture and processing sectors
Methodology:

- Risk Assessments
  - Risk management strategic framework and process applied to each industry sector
  - Identify and compile a risk library
  - Convene expert panel workshop (3rd October):
    - State & national disease, health & biosecurity specialists
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  - The expert panel will:
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## Risk analysis process:

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Key Outputs:

- Management strategies
  
  - Codes of practice for each industry sector
    - Synthesis of results from literature and expert reviews and the risk assessment (expert panel) workshop

- Disease outbreak simulations or training workshops for industry
Some key components:

- Close life cycle - Specific Pathogen Free
- Source & health status of new stock
- Quarantine of new & diseased stock
- Broodstock water treatment
- Regular health monitoring of stock-on farm
- Disposal of offal (processing wastes) and mortalities
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  - Trained staff

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  - Eg. Minimise / avoid stressful conditions

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Disease surveillance
The assessment of disease impact on wild abalone populations

- 18 regular monitoring sites surveyed last week 13-Sep (previous survey 26-Feb-06)
- 6 belt transects (30m x 1m) per site
  - All abalone counted into 3 size classes
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Monitoring of Crags (5 sites) to continue bi-monthly with all 18 sites to be re-surveyed in Jan-07
Survey locations
Crags sites

[Map showing the locations on the water of Mount Hummock and Boulder Point.]
Observations

- **All locations other than Craggs**
  - No signs of disease apparent
  - Only one (Passage Port Fairy) showed signs of recent mortality (empty shells > 1 month old)
  - Abundances were generally high
  - Wrasses abundant

- **Craggs**
  - Considerable number of dead abalone
  - Considerable number of moribund abalone lacking pedal adhesion
  - Absence of wrasses
  - Rock lobsters **not** feeding on dead abalone
  - Mucous and sloughed tissue prevalent in water column
Slipping from vertical surfaces
Falling from overhangs
Sub-legal abundance

Pre-recruits

Abundance/site

2000 2001 2002 2003 2004 2005 2006 2007

Year

Site #

129
130
131
132
133
Stock abundance

Recruits

Year

Abundance/site

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Epidemiological aspects of the herpes-like virus outbreak in Victoria

Dr Paul Hardy-Smith
Panaquatic® Health Solutions Pty Ltd
Acknowledgements

Help from the following has been gratefully appreciated:

• Victorian Department of Primary Industries
  – For commissioning the reports on which this presentation is based
  – For providing valuable assistance in compiling the information

• The farmed abalone sectors
  – Particularly farms that were affected

• The wild capture abalone sector – particularly Western Abalone Divers Association

• Gribbles Veterinary Pathology

• Tasmania Department of Primary Industries and Water

• The Australian Animal Health Laboratories (AAHL)
The affected area

VICTORIA

Portland
Farm 1
Farm 2
Port Fairy
Bass Strait

Processor
Melbourne

Port Phillip Bay
Westernport Bay

Farm 5
Unaffected

Flinders
Farms 3+4

Direct distance from Portland to Flinders is approximately 300km
Initial involvement

- Photos of affected abalone sent by manager of Farm #1 on 4th January, 2006
- Phone conversation with manager the following day
- Concurrently samples taken by Dr Celia Hooper (visiting farm #1 as part of research project)
- Pattern of spread and clinical signs ominous – toxic cause considered, discussed Electron Microscopy to rule out infectious agent
- Visited Farm #1 on January 10th, 2006
  - received call from Dr Judith Handlinger who had reviewed samples with Dr Hooper - concerned cause was infectious (and not bacterial or protozoal), suspicious inflammatory changes
  - ‘courtesy’ call made to Dr Mehdi Doroudi (DPI)
Initial alert

- At farm #1 worked into the night
  - Clinical aspects on both Farms
  - Epidemiology
- Infectious agent highly suspect due to:
  - numerous movements onto farms of wild abalone
  - epidemiological pattern of spread within farms
  - link between farms of live movement of abalone
- Hallmarks of viral disease
Disease confirmed

- Briefed farm staff – difficult!
- Conference call with Victorian DPI
  - Suspect highly infectious agent, viral
- Further samples collected from Farm#1 and delivered them to PIRVic Attwood that afternoon.
  - “Ganglioneuritis” confirmed at PIRVic Attwood (and Gribbles)
  - Presence of viral particles confirmed by Electron Microscopy at AAHL
The investigation

- Initially commissioned by DPI to provide an epidemiological report on the outbreak – completed January 26, 2006

- Subsequent report commissioned and completed August, 2006
The investigation

- This investigation is ongoing
- Both reports so far based on descriptive epidemiology*
  - i.e. description of the circumstances and factors (host, environment, time) leading to disease
- As much as possible attempted to identify the facts (that could be substantiated) of the outbreak

*Epidemiology can be defined as the study of patterns of disease that exist under field conditions (Martin et al, 1987)
The investigation

A few simple facts

- Four abalone farms affected in this outbreak
  - two pump ashore farms
  - two sea ranch style operations
- Wild abalone in the vicinity of one affected pump ashore farm affected
- Can only diagnose disease in abalone that have pathological changes (‘ganglioneuritis’)
- Greenlip (*H. laevigata*), blacklip (*H. rubra*) and crosses (‘hybrids’) confirmed affected
Best fit scenario?

Assumptions (based on current knowledge):
• Index case probably occurred on Farm #1
• Most likely came from wild stock
• When it was brought onto farm uncertain, but assume it was not there before November 18th, 2005
• Three farms affected linked by movement of live abalone
Other possible sources?

- Feed
- Birds
- Rodents
- People and equipment
- Pumped water
- Other gastropod molluscs
Conclusion

• We have a jigsaw puzzle with very few pieces
• As more pieces become available, the puzzle may change, and pieces may move
• Going forward, the development of more accurate tools highly desirable
• As noted by Renault et al (2000):

(Light microscopy)… appears poorly adapted to virus diseases and needs to be improved upon by other techniques such as transmission electron microscopy. Both techniques are time consuming and inadequate for epidemiological surveys.
Thank you
Way Forward

Dr Peter Appleford
Executive Director Fisheries Victoria
21 September 2006
National Approach

- Evidence and analysis based
Disease Response

- Active surveillance
- Disease control strategies
- Compensation
Stock Management

- Stop harvest in interim
- Determine stock status
- Reintroduce harvesting – sustainably
Biosecurity

- Need good biosecurity
- Aquaculture
- Wild harvest
- Processors
- Recreational
- In line with National translocation guidelines
Knowledge

- Fill knowledge gaps – science/research
Focus on OsHV-1, a virus infecting bivalves

By T. Renault
Laboratoire de Génétique et Pathologie
17390 La Tremblade
France

Ifremer
What we have done

Description in the French Pacific oyster (diseased larvae and seed)

Pathogenicity and transmissibility (experimental infections)

Viral purification (DNA extraction, cloning, sequencing...)

Development of diagnosis tools

Characterisation of the viral genome
Disease manifestations

- Viral infections described in larvae and spat (juveniles) in association with high mortality rates (90% to 100% in larvae, 50% to 80% in juveniles) since 1991 in France

Velar lesions (arrow) of a Crassostrea gigas larva infected with OsHV-1
Disease manifestations

- No virus infection reported in adult oysters
- However, OsHV-1 was detected in French asymptomatic *C. gigas* adults using molecular and immunological tools
Descriptive histopathology

- Lesions confined to connective tissue (mantle, gonads, gills and digestive gland in spat)
- Abnormal cytoplasmic basophilia and enlarged nuclei with chromatin margination

Semithin section of a *Crassostrea gigas* larva infected with OsHV-1
Descriptive histopathology

- Abnormal cytoplasmic basophilia and enlarged nuclei with chromatin margination also detected in the adductor muscle, the heart and the visceral ganglia.

*In situ* hybridisation: confirmation of the presence of *OsHV-1* in heart and visceral ganglia.
Experimental transmission

- Experimental transmission to axenic C. gigas larvae, associated with high mortality rates and detection of viral particles (fulfill Koch's postulates)

Pathogenicity of the virus infecting C. gigas larvae demonstrated
Interspecies transmission

- Experimental transmission to bivalve larvae belonging to different species, associated with high mortality rates and viral detection (TEM, PCR and sequence analysis)
Interspecies transmission

Crassostrea gigas

Crassostrea angulata

Crassostrea ariakensis

Ostrea edulis

Ruditapes philippinarum

Pecten maximus

Crassostrea gigas
Interspecies transmission

- OsHV-1 confined to a single host species in nature?
- Does intensive farming conditions promote transmission to new host species (different bivalves species in large numbers in unnaturally close proximity)?
OsHV-1 ultrastructure

Icosahedral capsid (72-75 nm)

Envelope (90-120 nm)

Core

OsHV-1 particles are similar in overall appearance to those of other herpesviruses
OsHV-1 genome

Viral genome extracted from purified particles (naturally infected C. gigas larvae): linear ds DNA and total genome size: 207 kbp (herpesviruses: 100-250 kbp)

The sequence data demonstrate that OsHV-1 is not closely related to herpesviruses with vertebrate hosts (including fish)
Evolution and taxonomy of OsHV-1

Herpesviruses of mammals and birds, herpesviruses of fish and amphibians and herpesviruses of invertebrates form three major lineages of the herpesviruses.
Evolution and taxonomy of OsHV-1

The oyster herpesvirus is the first identified member of the third major domain of the Herpesviridae.

The herpesvirus infecting C. gigas oyster was named Oyster Herpesvirus 1 (OsHV-1): the single representative of invertebrate herpesviruses.
Transmission assays

Experimental infection of axenic larvae

- Oshv-1 infected larvae
- Grinding in seawater
- Vortexing and centrifugation
- Addition of 10 ml seawater
- Filtering (0.45 µm and 0.22 µm)
- Mock-infected larvae: inoculation of sterile seawater (× 3 containers)
- 3 day-old axenic larvae

Analysis
Transmission electron microscopy
Larvae or seed were ground in double distilled water (1 g/ml) and denatured in a boiling water bath for 10 minutes. Samples were then centrifuged at 2000 g for 5 minutes.
PCR analysis

An epidemiological survey in France from 1997 to 2005
In situ hybridisation

1. Dewaxing + Proteinase K
2. Denaturation (94°C) Hybridisation Overnight (42°C)
3. Blocking Agent
4. Incubation Washes
5. Incubation Washes
6. Revelation
7. Anti-Dig Antibody
8. Hybridisation Buffer + Dig Labelled Probe
9. Digoxigenine Labelled Probe (PCR)
Immunological detection

Histological section of OsHV-1 infected Crassostrea gigas spat
Detection of infected cells using murine specific polyclonal antibodies (heart)
Diagnosis tools

- No cell line available: to date, all assays using different cell lines (fish, insect, mammal and BGE cell lines) failed.
Thank you very much for your attention