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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 1 – Documents 1-40

For more information, please refer to CSIRO's FOI disclosure log at www.csiro.au/FOILog

1

From: Crane, Mark (LI, Geelong)
Sent: Tuesday, 17 January 2006 11:09 AM
To: Hyatt, Alex (LI, Geelong)
Cc: GA - Fish Diseases Laboratory; Walker, Peter (LI, Geelong); Daniels, Peter (LI, Geelong); Jeggo, Martyn (LI, Geelong)
Subject: Mortality of 2 Victorian Abalone farms

Follow Up Flag: Follow up
Flag Status: Completed

Alex,

Malcolm Lancaster (VIAS) called concerning on-going mortalities at two Abalone farms. Their investigation of the mortalities has shown histopathology consistent with that reported from mortalities in Taiwan associated with a herpes-like virus (see attached pdf). There has been movement of stock between the two farms so there is an epidemiological connection. Malcolm is sending fixed ganglion material for examination by EM - it should arrive tomorrow. Apart from EM there is not much more we can do at this stage. If there is indeed a herpes-like virus then we can discuss if any further work needs to be undertaken.

Cheers

Mark

Abalone
pes-like virus DAC

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Vol. 65: 23–27, 2005 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published June 14

Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan

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ABSTRACT: A herpes-like virus is demonstrated for the first time to be associated with high mortality rates in maricultured abalone *Haliotis diversicolor supertexta* in Taiwan. Histopathology of moribund abalone indicated that the nerve system was the primary target tissue. The lesions were characterised by tissue necrosis accompanied with infiltration of haemocytes. Electron microscopic examination demonstrated viral particles within the degenerated cerebral ganglion cells. The viruses were hexagonal, approximately 100 nm in diameter and had a single coat. Some viral particles contained a dense nucleoid, while others were empty. The ultrastructure and morphogenesis of the virus particles were consistent with those of the herpesvirus described from the oyster *Crassostrea virginica*. Experimental infection using supernatant collected from minced visceral organs and muscle of moribund abalone induced 100% mortality through both intramuscular injection and bath treatments.

KEY WORDS: Herpes-like virus · *Haliotis diversicolor supertexta* · Mortality · Pathogenicity

INTRODUCTION

Herpes-like virus infection in marine bivalves was first observed in adult oysters *Crassostrea virginica* (Farley et al. 1972). Subsequently, this virus has been detected in other oyster species (Hine et al. 1992, Nicolas at al. 1992, Comps & Cochennec 1993, Renault et al. 1994, 2000). Herpesviruses have also been characterised in nonostreid bivalves such as European clam *Ruditapes decussatus* and Manila clam *R. philippinarum* (Renault & Arzul 2001, Renault et al. 2001). Among these viruses, ostreid herpesvirus-1 (OsHV-1) (Minson et al. 2000) and a variant of OsHV-1 (OsHV-1 var) have been analysed by PCR (polymerase chain reaction), restriction endonuclease digestion of PCR products and DNA sequencing. OsHV-1 and OsHV-1 var are representatives of a single viral species that may be the ubiquitous cause of herpesvirus infections in marine bivalves (Arzul et al. 2001).

Abalones are members of the family Haliotidae and the genus *Haliotis*, having 1-piece shells. Mass mortality of the abalone associated with *Vibrio carchariae* and withering syndrome have been described (Gardner et al. 1995, Nicolas et al. 2002). The aetiological agent of withering syndrome has been identified as a unique taxon, and the provisional status of *Candidatus xenohaliotis californiensis* has been proposed (Friedman et al. 2000). However, the mortality of

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abalone caused by herpes-like viruses has not been reported.

The abalone *Haliotis diversicolor supertexta* is a dominant gastropod mollusc used for mariculture in Taiwan. Farms are located in coastal areas around the island. The annual product value of Taiwan abalone exceeds US$200 million, including US$58.8 million from those raised in farms along the northeast coast. In January 2003, high mortality of abalone occurred among both land-based and ocean-based ponds in north eastern Taiwan that resulted in losses of US$11.5 million to the domestic abalone industry. We describe here for the first time a herpes-like virus that infects abalone cultured in Taiwan.

MATERIALS AND METHODS

Animals. Moribund abalone *Haliotis* spp., 5 to 6 cm in shell length, were collected from both land-based and ocean-based farms experiencing increasing mortality on the northeast coast of Taiwan in March 2003. Bacterial isolation was performed from the hepatopancreas, gonad and muscle by inoculating tissue onto Trypticase Soya agar (TSA, Difco) plates and incubating them at 30°C for 24 h.

Histopathology. Samples of visceral organs, muscle and mantle were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 µm and stained with Mayer's haematoxylin and eosin (H&E).

Electron microscopy. Two different techniques were applied. For thin-section electron microscopy of paraffin-embedded tissues, cerebral ganglions were retrieved from the paraffin block, deparaffinised and reprocessed for electron microscopy. Then, 1 ml of 2.5% glutaraldehyde (0.1 M cacodylate buffer, pH 7.2) was added to the samples, and they were fixed for 6 h; then they were rinsed and equilibrated 3 times for 10 min each using 0.1 M cacodylate buffer. Osmium tetroxide (0.1 M cacodylate buffer, pH 7.2) at 1% was carefully added to the samples, and they were kept at room temperature for 2 h. Samples were rinsed and equilibrated 3 times for 10 min each using 0.1 M cacodylate buffer, then dehydrated using a gradient series of ethanol at 35, 50, 70, 85, 90, 95 and 100%, for 10 min each. Samples were immersed in a series of ethanol-resin mixtures for 2 h each. The ratio of ethanol to resin was changed from 3:1 to 1:1 to 1:3. Samples were embedded by 100% resin immersion. Then, samples were polymerised for 24 h at 70°C. Thick sections were stained with 1% toluidine blue solution and observed under a light microscope to determine cell morphology. Ultrathin sections (0.35 mm) were double-stained with 1% uranyl acetate and lead citrate and examined using transmission electron microscopy.

For negative-contrast electron microscopy, samples of visceral organs, muscle and mantle of 10 moribund abalone were pooled, homogenised in phosphate-buffered saline (PBS) to give a 10% (w/v) suspension, and centrifuged at 3000 × *g* for 20 min at 4°C. The supernatant was centrifuged at 100 000 × *g* for 10 min; the pellet was negatively stained with 2% phosphotungstic acid and examined using electron microscopy (Bozzola & Russell 1992, Lipart & Renault 2002).

Preparation of virus solution for the infection trail. Abalone collected from a farm was used for the study. Tissues were removed from moribund abalone, minced, resuspended in MEM and homogenised for 1 to 2 min in a Virtis blender. The homogenate was clarified by centrifugation at 1500 × *g* for 20 min at 4°C. The supernatant fluid was collected and passed through 0.45 mm membrane filters.

Infection trial. The susceptibility of abalone to herpes-like viruses was examined following experimental exposures with the viral supernatant. Three groups of 10 abalone each obtained from a farm in southern Taiwan that had not experienced an epizootic were divided into herpes-like-virus and control groups. Experiments were carried out in 100 l glass aquaria containing 70 l of salt water. Aquaria were aerated by water recirculating through in-tank, glass-wool filters at 17 to 20°C. In the injection group, 10 abalone were intramuscularly (IM) injected with 0.1 ml of supernatant. In the bath group, 10 abalone were bathed in a 500× diluted supernatant for 30 min, and then stocked in separate aquaria. Two control groups were treated by both injection and bathing following the same procedures, but using MEM instead. Mortalities of abalone were recorded daily. Duplicate infection trials have been carried out in this study.

RESULTS

Epidemiological investigation

Commencing in January 2003, mass mortalities of *Haliotis* spp. occurred among cultured abalone in north eastern Taiwan. During the epizootic, the water temperature was 16 to 19°C. Both adult and juvenile abalone suffered from the disease, with cumulative mortalities of 70 to 80%. Death of all of the abalone in a pond can occur within 3 d of the onset of clinical signs. The disease was reported only in *H. diversicolor supertexta*, while cohabitating Japanese black abalone *H. discus* remained normal. The gross appearance of affected abalone revealed mantle recession and muscle stiffness.

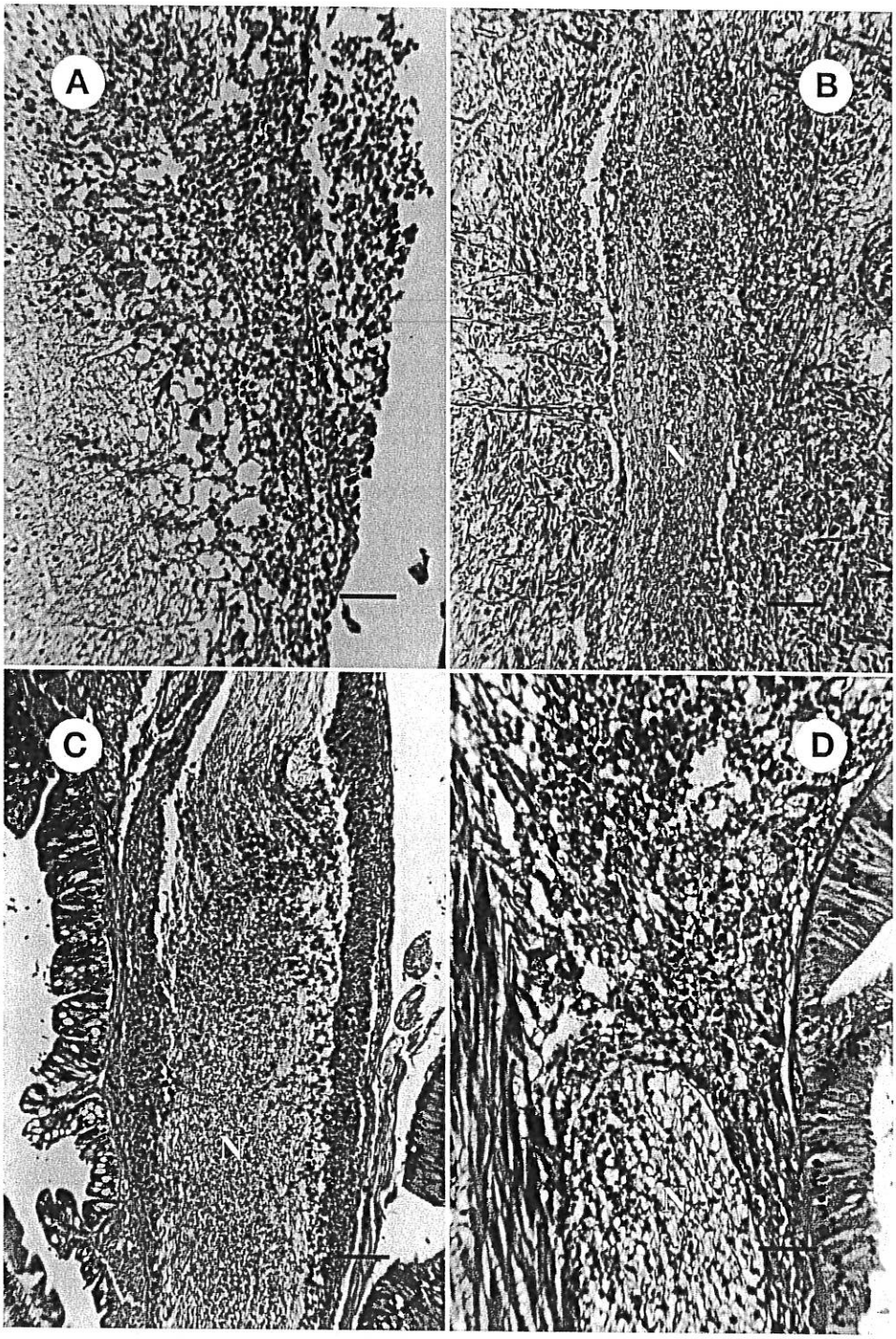

Fig. 1. *Haliotis diversicolor supertexta*. (A) Histological examination of the cerebral ganglion revealing lesions in the cerebral ganglion and surrounding neurilemma. Diffuse necrosis accompanied with infiltration of haemocytes was prominent in the tissue. Degenerated neurosecretory cells (arrow) were noted. Scale bar = 50 µm. (B) Histological examination of the foot revealed lesions in the nerve (N) and surrounding muscular tissue. Diffuse necrosis accompanied with infiltration of haemocytes was evident. Scale bar = 100 µm. (C) Histological examination of the oesophagus revealed lesions in the nerve (N) beneath the mucosa. The lesions involved the nerve and surrounding neurilemma. Scale bar = 100 µm. (D) Histological examination of the intestine revealed lesions in the muscle and adjacent nerve tissue (N) beneath the mucosa. The lesions consisted of diffuse necrosis accompanied with infiltration of haemocytes. Scale bar = 50 µm

Histopathology

Histological examination of moribund abalone indicated that the nerve system was the primary target tissue. The lesions were characterised by tissue necrosis accompanied with infiltration of haemocytes. Lesions in the cerebral ganglion consisted of diffuse necrosis and prominent infiltration of haemocytes in the parenchyma and surrounding neurilemma (Fig. 1A). Lesions involving nerve tissues and the surrounding muscular tissue in the foot were evident (Fig. 1B). Similar lesions were also noted in the oesophagus (Fig. 1C) and intestine (Fig. 1D). In some cases, diffuse necrosis accompanied with infiltration of haemocytes in the epithelia and underlying lamina propria of branchial filaments was evident. The sloughing off epithelia were deposited in the interlamellar spaces.

Electron microscopy

TEM examination of cerebral ganglions demonstrated viral particles within the degenerated cells (Fig. 2A). The viral particles were hexagonal, 90 to 100 nm in diameter and had a single coat. Some particles contained a dense nucleoid, while others were empty (Fig. 2B). The morphology and size of the viral particles were most consistent with a herpes-type virus described from the oyster *Crassostrea virginica* (Farley et al. 1972). Negative-contrast electron microscope examination demonstrated viral particles in the pooled tissue. The viral particles were hexagonal, 90 to 100 nm in diameter and had a single coat.

Infection trial

Abalone died at 2 dpi (days post-inoculation) and 3 dpi in the bath treatment, while all abalone died at 2 dpi with the IM-injection treatment. No abalone died in the control groups. Experimentally infected abalone in both IM-injection and bath groups developed lesions similar to those of naturally affected abalone. No macroscopic or microscopic lesions were observed in the 2 noninfected controls. A duplicate infection trail induced similar mortality and pathology in both treatments.

DISCUSSION

Herpes-like virus infections in abalone have not previously been reported. We now describe for the first time a herpes-like virus infection of maricultured

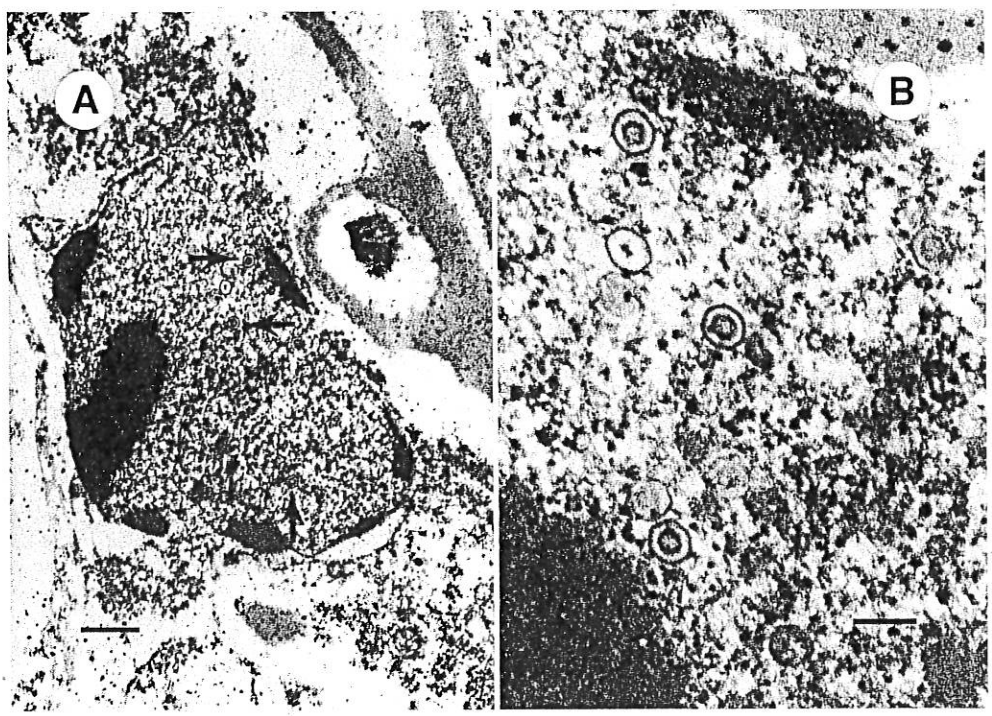

Fig. 2. *Haliotis diversicolor supertexta*. (A) TEM examination of cerebral ganglions showing viral particles (arrows) within degenerated cells. Scale bar = 416 µm. (B) The viral particles were hexagonal, 90 to 100 nm in diameter and had a single coat. Scale bar = 125 µm

abalone *Haliotis diversicolor supertexta* in association with high mortality rates. As the pathology specific to herpes-like virus infection has not been described and no abalone cell line is available to facilitate virus culture, infections were confirmed by detection of virus particles in the lesions and by infection trail.

The herpes-like virus detected in moribund abalone is a cytocidal virus, with necrosis as its principal pathological effect. The virus caused fatal disease, and exhibited a high degree of neurotropism. Nervous system involvement, affecting the cerebral ganglion and nerve bundles in the muscle of the foot as well as the muscular layers beneath the visceral organ, was noted, although it also induced lesions in other tissues.

Virus replication associated with tissue necrosis in the cerebral ganglion was demonstrated by TEM examination of paraffin-embedded tissues. The morphology and size of the virus particles were similar to those documented previously in the oyster (Farley et al. 1972, Roizman & Baines 1991). The morphological form corresponding to replicating viruses was noted at an intranuclear location. Two classes of nucleocapsids were observed: one with an electron-dense core, which corresponds to DNA-containing capsids, and the other, which lacked the core. Enveloped capsids (virions) were not observed in the cytoplasm, due to destruction of cellular organisms by fixation.

The infection trail following IM-injection and bath treatments demonstrated the high degree of virulence by inducing 100% mortality in abalone. These results demonstrate that the virus induced mortalities and could be transmitted horizontally from infected to healthy abalone using the bath treatment. A pathology similar to that of the field cases was reproduced in the infection trail, and further confirms the tropism of herpes-like viruses for nerve tissues.

The focal origin, high mortality rates and lack of previous events of comparable magnitude suggest that the disease agent was probably an exotic pathogen. It is speculated that this virus was introduced through the importation of juvenile abalone, because a farmer had introduced juvenile abalone from abroad prior to this epizootic. During the epizootic, the highest prevalence was observed during the winter months. The prevalence dropped in the spring and summer periods, possibly because heavily infected individuals died in the winter, and the disease did not begin another cycle of increasing infection until autumn.

Editorial responsibility: Jo-Ann Leong, Kaneohe, Hawaii, USA

Acknowledgements. This study was supported by a grant from the Council of Agriculture, ROC.

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Submitted: February 18, 2004; Accepted: December 23, 2004
Proofs received from author(s): May 24, 2005

DOCUMENT 2

EXEMPT IN FULL – s 37(1)(b)

From: Crane, Mark (LI, Geelong)
Sent: Wednesday, 25 January 2006 4:44 PM
To: 'Mehdi.Doroudi@dpi.vic.gov.au'
Cc: Jeggo, Martyn (LI, Geelong); Daniels, Peter (LI, Geelong); Walker, Peter (LI, Geelong); Hyatt, Alex (LI, Geelong); GA - Fish Diseases Laboratory
Subject: Abalone Incident: Preliminary results

Dear Mehdi,

As promised here are AAHL's preliminary findings on the submitted Abalone samples:

Alex Hyatt has undertaken preliminary examination by electron microscopy and has observed herpes-like virus particles (based on size and morphology) in the nucleus of host cells. Examination will continue in an attempt to observe virus particles budding through the nuclear membrane and in the cytoplasm.

A full and formal diagnostic report will be prepared following completion of the examination by electron microscopy.

Regards

Mark

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email: mark.crane@csiro.au

4

From: Malcolm.Lancaster@dpi.vic.gov.au
Sent: Friday, 27 January 2006 3:38 PM
To: Crane, Mark (LI, Geelong); Mehdi.Doroudi@dpi.vic.gov.au
Subject: abalone disease transmission trial draft
Attachments: INVESTIGATION OF ABALONE GANGLIONEURITIS.doc

Follow Up Flag: Follow up
Flag Status: Completed

Mark, Mehdi:

Attached is a first draft.

Malcolm Lancaster

INVESTIGATION OF ABALONE GANGLIONEURITIS

INTRODUCTION/BACKGROUND

High mortality rate seen on two abalone farms in Victoria, with fewer deaths on a third Victorian farm. Abalone transferred from one of the farms to the other two farms prior to increased mortality rate.

Species affected:

Haliotis laevigata,
Haliotis rubra
Haliotis laevigata x *rubra* hybrids

Clinical signs
Depression, inappetence, radula out of mouth

Histopathology
Ganglioneuritis – infiltration of haemocytes in multiple ganglia and nerves.
Cerebral ganglion affected, also other ganglia and nerves.

Electron microscopy
Virus visualized in pleuropedal ganglion (Alex Hyatt)

Report of cerebral ganglioneuritis in *Haliotis diversicolor* associated with a herpes-like virus in Taiwan in 2003 (Chang PH et al 2005).

AIMS

1. Characterisation of virus

Reported only as "herpes-like" (Chang PH et al 2005).
Concentrate virus for further electron microscopic examination.
Extract DNA from concentrated virus preparation and PCR to confirm as a herpesvirus.

2. Fulfilment of Koch's postulates

Concentrate virus, filter, inject into naïve animals, reproduce clinical disease and confirm presence of virus in injected animals away from the injection site.

3. Confirmation of infectious nature of disease

Demonstration of transmission of clinical disease by cohabitation of sick abalone and naïve abalone.
(The injection trials may fail, and the visualized virus may be a commensal.)

MATERIALS AND METHODS

1. Characterisation of virus

Collect as many infected abalone from CSF as possible.

Freeze in dry ice immediately, or,.
Deliver live to laboratory

Dissect out nervous tissue.
Concentrate virus by density gradient centrifugation of macerated tissue

Negative contrast electron microscopy
Chang PH et al (2005)
samples of visceral organs, muscle and mantle of 10 moribund abalone were pooled, homogenised in phosphate buffered saline (PBS) to give a 10% (w/v) suspension, and centrifuged at 3000 x g for 20 min at 4'C. The supernatant was centrifuged at 100000 x g for 10 min; the pellet was negatively stained with 2% phosphotungstic acid and examined using electron microscopy

2. Fulfilment of Koch's postulates

Preparation of virus solution for the infection trial
Chang PH et al (2005)
Tissues were removed from moribund abalone, minced, resuspended in MEM and homogenised for 1 to 2 min in a Virtis blender. The homogenate was clarified by centrifugation at 1500 x g for 20 min at 4'C. The supernatant fluid was collected and passed through 0.45 mm membrane filters.

Infection trial.
Chang PH et al (2005)
Three groups of 10 abalone

Experiments were carried out in 100 1 glass aquaria containing 70 1 of salt water, Aquaria were aerated by water recirculating through in tank, glass wool filters at 17 to 20'C.

In the injection group, 10 abalone were intramuscularly (IM) injected with 0.1 ml of supernatant.

In the bath group, 10 abalone were bathed in a 500x diluted supernatant for 30 min, and then stocked in separate aquaria.

Two control groups were treated by both injection and bathing following the same procedures, but using MEM instead.

Mortalities of abalone were recorded daily.

3. Confirmation of infectious nature of disease

Add sick abalone to tank of healthy naïve abalone

Or leave uninjected healthy naïve abalone in tank with injected abalone.

References

Chang PH et al (2005) Chang PH, Kuo ST, Lai SH, Yang HS, Ting YY, Hsu CL, Chen HC. Herpes-like virus infection causing mortality of cultured abalone Haliotis diversicolor supertexta in Taiwan. Dis Aquat Organ. 2005 Jun 14;65(1):23-7.

From: Crane, Mark (LI, Geelong)
Sent: Monday, 30 January 2006 1:24 PM
To: 'Malcolm.Lancaster@dpi.vic.gov.au'
Subject: RE: abalone disease transmission trial draft

Follow Up Flag: Follow up
Flag Status: Completed

Categories: NQS

That's correct, Malcolm - We don't have any abs at the moment.

Mark

MARK CRANE Ph.D.
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email: mark.crane@csiro.au

-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Monday, 30 January 2006 13:20
To: Crane, Mark (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au; Corbeil, Serge (LI, Geelong)
Subject: RE: abalone disease transmission trial draft

Mark:

When "they" were talking about an AqCCEAD at the end of last week, it was to be held after they received your official report. So presumably they will be swinging into action shortly.
There is a possibility that we may receive fresh abalone material from Abanex (off Flinders) today or tomorrow - we will freeze it down at -80 degrees C if that is the case, as I assume you won't have abs in tanks at AAHL ready.

Malcolm

Mark.Crane@csiro.au

30/01/2006 01:09 PM

To: Malcolm.Lancaster@dpi.vic.gov.au, Mehdi.Doroudi@dpi.vic.gov.au
cc: Serge.Corbeil@csiro.au
Subject: RE: abalone disease transmission trial draft

Thanks, Malcolm.

Serge and I will review this and get back to you. I think we all agree that the top priority is to attempt transmission experimentally with fresh and frozen material and to freeze virus down under the most

appropriate conditions. Hopefully frozen virus will remain viable and this will allow us time to develop a research proposal for FRDC.

I presume the finding of a virus will prompt an AqCCEAD and other options for further work (and funding) may de discussed then.

Cheers

Mark

MARK CRANE Ph.D.
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-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Friday, 27 January 2006 15:38
To: Crane, Mark (LI, Geelong); Mehdi.Doroudi@dpi.vic.gov.au
Subject: abalone disease transmission trial draft

Mark, Mehdi:

Attached is a first draft.

Malcolm Lancaster

6

From: Crane, Mark (LI, Geelong)
Sent: Monday, 30 January 2006 1:04 PM
To: Hugh.Millar@dpi.vic.gov.au; 'Bronwyn.Murdoch@dpi.vic.gov.au'; Mehdi.Doroudi@dpi.vic.gov.au; 'Malcolm.Lancaster@dpi.vic.gov.au'
Subject: RE: abalone Final Report
Attachments: 06-00208.pdf

Follow Up Flag: Follow up
Flag Status: Completed

Dear All,

Please see attached Final Report. Hard copies are in the mail.

Regards

Mark

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Australian Animal Health Laboratory (AAHL)

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Diagnostic Specimen Testing

Final Report

(PIRvic) Primary Industries Research
Attn: Bronwyn Murdoch
475 - 485 Mickleham Road
Attwood Victoria 3049
AUSTRALIA

SAN : 06-00208
Date : 30-JAN-2006

Sender	Bronwyn Murdoch (PIRvic) Primary Industries Research	**Ref :** 2006-000112
Owner	Coastal Seafarms Portland VIC AUSTRALIA	
Examination requested	Virus EM	
Sample Information as provided	Portland, Victoria	
Sample Collection Date	12-JAN-2006	**Received at AAHL** 18-JAN-2006

Electron Microscopy Results

Test Type: Electron Microscopy Date Tested: 30 Jan 2006

No.	Specimen ID	Specimen Description	Test Result
2	1 2 3 18 20 5 7 9	Abalone - Fixed tissue Not Specified	Herpes like virus detected

Comment(s): Ultrathin sections from four blocks were examined. Of these viral particles were observed within three sections. The viruses were present in necrotic and what appeared to be health nuclei of the ganglia. The viruses were icosahedral with electron dense cores. The diameter was 104nm 4 (n=17). The size of the naked viruses is characteristic of herpesviruses as is the nuclear location and varied nature of the electron dense cores. One particle was observed external to the nucleus. The ultrastructure of the viruses was similar to that described by Chang et al (Herpes-like virus infection causing mortality of cultured abalone Haliotis diversicolor supertexta in Taiwan), Diseases of Aquatic Organisms, vol63: 23-27, 2005.

Yours faithfully

Axel Colling
Authorised Signatory

7

From: Crane, Mark (LI, Geelong)
Sent: Monday, 30 January 2006 11:54 AM
To:
Cc: Corbeil, Serge (LI, Geelong); Mehdi Doroudi (mehdi.doroudi@dpi.vic.gov.au)
Subject: RE: Abalone Incident: Preliminary results

Follow Up Flag: Follow up
Flag Status: Completed

Hi

Good to hear from you. Yes, it was a good result - Alex did note that the particles were fairly scarce and he had to do a bit of searching. With respect to the PCR, that is something we have discussed and have yet to do.

Cheers for now

Mark

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DELETION

s22

-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Thursday, 26 January 2006 1:34 PM
To:
Cc: bronwyn.murdoch@dpi.vic.gov.au
Subject: Abalone Incident: Preliminary results

Hi Celia, Tony:

We prepared abalone tissues for em examination here, but as we didn't see any viral particles ourselves, we took material to AAHL and Alex Hyatt. His interim (unofficial, not for promulgation) findings are below.

Cheers,
Malcolm

----- Forwarded by Malcolm Lancaster/NRE on 26/01/2006 01:27 PM -----

Mehdi Doroudi

25/01/2006 08:11 PM

To: Hugh Millar/NRE@NRE, Peter Appleford/NRE@NRE, Anthony Forster/NRE@NRE, Alison Lee/NRE@NRE, Malcolm Lancaster/NRE@NRE
cc:
Subject: Abalone Incident: Preliminary results

FYI

Mehdi Doroudi - DVM, PhD
Research Director
Marine & Freshwater Systems - PIRVic
Department of Primary Industries

Ph: +613 5258 0272 Fax: +613 5258 0270
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2A Bellarine Hwy, Queenscliff VIC 3225 Australia
PO Box 114, Queenscliff VIC 3225 Australia

----- Forwarded by Mehdi Doroudi/NRE on 25/01/06 08:09 PM -----

Mark.Crane@csiro.au

25/01/06 04:43 PM

To: Mehdi.Doroudi@dpi.vic.gov.au
cc: Martyn.Jeggo@csiro.au, Peter.Daniels@csiro.au, Peter.Walker@csiro.au, Alex.Hyatt@csiro.au, afdl@csiro.au
Subject: Abalone Incident: Preliminary results

Dear Mehdi,

As promised here are AAHL's preliminary findings on the submitted Abalone samples:

Alex Hyatt has undertaken preliminary examination by electron microscopy and has observed herpes-like virus particles (based on size and morphology) in the nucleus of host cells. Examination will continue in an attempt to observe virus particles budding through the nuclear membrane and in the cytoplasm.

A full and formal diagnostic report will be prepared following completion of the examination by electron microscopy.

Regards

Mark

MARK CRANE Ph.D.
Project Leader
AAHL Fish Diseases Laboratory
Australian Animal Health Laboratory
CSIRO Livestock Industries
Private Bag 24
Geelong Vic 3220

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International Fax: ⅄3 52 275555
email: mark.crane@csiro.au

Attention:

DOCUMENT 8

EXEMPT IN FULL – s 47B(a)

From: Crane, Mark (LI, Geelong)
Sent: Wednesday, 1 February 2006 11:20 AM
To: Corbeil, Serge (LI, Geelong); Williams, Nette (LI, Geelong)
Cc: Edwards, Steven (LI, Geelong)
Subject: Saltwater aquarium

Follow Up Flag: Follow up
Flag Status: Completed

Hi both,

ABALONE EXPERTISE
I have spoken with Nathan from Queenscliff concerning abalone facilities and, being a finfish expert, he could not really provide much information. In addition, since he is working with fish at MAFRI he could not visit the secure facility here.

Thus I contacted Steve Rodis at Great Southern Waters at Indented Head and he is happy to provide us with information but suggested that we (with Mehdi) visit his farm to take a look at the facility and to discuss how we can adapt our research aquarium facility for abalone. I have spoken with Mehdi and he has agreed to do this next week (date yet to be confirmed). I know that Serge has to go secure each day and therefore could not visit the farm. Nette: After today is it possible for you to stay non-secure if you are interested in a visit to GSW?

AAHL AQUARIUM
Steve Edwards has started his evaluation of the saltwater system here at AAHL. Level 5 seems to be functioning OK. There is some crud in the 1000L tank that needs to be cleaned out and I said that we would take care of that. The pump on level 5 seems to be working. When you visit the LAF this afternoon can you please confirm that there is pressure in the saltwater supply line in C8?

However, the in-flow of water to the level 5 tank seems to be very slow. Steve is going to check the pump on the outside by the 15 000L tank to see what the story is there. There may be some crud partially blocking the supply. Once this checks out the system will be flushed with freshwater and then we need to get saltwater in.

SUPPLY OF SALTWATER
I have sent an email to Mehdi asking how we can access saltwater from MAFRI now that they have relocated. Currently, Mehdi is visiting the infected farms in Portland with Hugh Millar and will get back to me on this issue when he returns to the office - probably Friday. It is clear to Mehdi that if we are to do this right we need to take our time and make sure that everything is in place to give it every chance. Industry knows that we will be doing these trials and so we need to make sure we conduct them the best way possible with the available resources (cf TAB studies).

STEVE RODIS' RESPONSES TO MY QUESTIONS

Hi Mark,

There are a lot of what ifs in there.

I need to know the number and size of animals you are considering.

If I understand it correctly you will be trying to replicate as closely as possible grow-on conditions that stock would normally be subject to.

Flow rate could be obtained by use of a small aquarium or fountain pump. You would need a cache of water to draw from and the size of this will be a direct result of stocking size and density. A normal gravel bed filter as used in aquariums could work to help strip the ammonia out of the water but it takes some time to build up the correct bacteria for this to work as anything more than a sediment trap.

Current temps could be expected in the 18-22 range (SOM would be cooler than CSF)

Feed is just manufactured feed and easy to handle.

It might be worthwhile you and Medhi coming down to GSW for a quick run through.

Regards,

Steve Rodis

We need to prepare a set of questions we need answers for prior to the visit to GSW. The things I asked Steve were:

We believe that the transmission trial will take up to 2 weeks to run. Therefore we need to maintain the abalone for a relatively short time period. What I need to know is what is the minimal conditions we can maintain abalone at:

Water flow rate? - For a two week period do we need water flow or can we use aerators and undertake regular water exchanges, when necessary.

Temperature range - I suspect we will run the experiment at the water temperature of the farms experiencing the mortalities.

Feed? - For the two weeks would we need to feed and if so what with.

Abalone density - we estimate that we need 8 experimental groups. We have 120 litre tanks - how many abalone can we put in each tank and what type of substrate do we need?

Are there any other factors that we need to be aware of for this short time period?

Appreciate any assistance/advice you can give.

MARK CRANE Ph.D.
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From: Malcolm.Lancaster@dpi.vic.gov.au
Sent: Friday, 3 February 2006 2:31 PM
To: Crane, Mark (LI, Geelong)
Subject: RE: herpeslike virus

Follow Up Flag: Follow up
Flag Status: Completed

Water temperature at the start of the outbreak was 17 degrees C
One year old stock have suffered the highest mortality. Young abalone (<10mm) have not been affected.

Mark.Crane@csiro.au

03/02/2006 09:29 AM

To: Malcolm.Lancaster@dpi.vic.gov.au
cc: mehdi.doroudi@dpi.vic.gov.au
Subject: RE: herpeslike virus

Hi Malcolm,

No - we are not ready. We are in the process of re-starting up our salt water system which will take a few days (we have had to replace a pump) and Mehdi and I were going to go to Great Southern Waters next week to find out about rearing conditions. Mehdi was going to get back to me about dates to visit GSW.

What I need to know from PirVic is:

Water temperature at which disease was precipitated
Size of abalone that we can expect - this will help to determine stocking rate and total numbers we can accommodate etc
Also we need to truck some water in from MAFRI, Queenscliff and I am not sure whether this facility is still available to us

We will have 8 experimental groups but do not know how many abalone per group until we know their size and have met with Steve Rodis at GSW to discuss how we can adapt our system to holding abalone.

Apologies for the delay but we do not have turn-key operation here and we need to get it right before we start.

Cheers

Mark

MARK CRANE Ph.D.
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email: mark.crane@csiro.au

-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Friday, 3 February 2006 07:58
To: Crane, Mark (LI, Geelong)
Subject: herpeslike virus

Hi Mark:

Mehdi will be at the abalone farm today - are you in a position to receive any samples yet?

Cheers,
Malcolm

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From: Mehdi.Doroudi@dpi.vic.gov.au
Sent: Saturday, 4 February 2006 12:03 PM
To: Crane, Mark (LI, Geelong)
Cc: Malcolm.Lancaster@dpi.vic.gov.au; Corbeil, Serge (LI, Geelong)
Subject: RE: abalone disease transmission trial draft

Follow Up Flag: Follow up
Flag Status: Completed

Mark,

I have provided Malcolm with more samples of sick abalone on last Friday. I will see you Tuesday afternoon to further discuss the ways forward.

Regards

Mehdi Doroudi - DVM, PhD
Research Director
Marine & Freshwater Systems - PIRVic
Department of Primary Industries

Ph: +613 5258 0272 Fax: +613 5258 0270
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PO Box 114, Queenscliff VIC 3225 Australia

Mark.Crane@csiro.au

03/02/06 10:59 AM

To: Malcolm.Lancaster@dpi.vic.gov.au, Mehdi.Doroudi@dpi.vic.gov.au
cc: Serge.Corbeil@csiro.au
Subject: RE: abalone disease transmission trial draft

Hi All,

This is a good outline for an in-depth study which our organisations may undertake as funding becomes available.

The most urgent need is to get some material into naive abalones to:

- Fullfill Koch's postulates
- To determine whether stored material is viable
- To generate experimental material for future research

If we can do this we will be doing well and the rest can follow.

As soon as Mehdi gets back from Portland we can discuss further

Cheers

Mark

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email: mark.crane@csiro.au

-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Friday, 27 January 2006 15:38
To: Crane, Mark (LI, Geelong); Mehdi.Doroudi@dpi.vic.gov.au
Subject: abalone disease transmission trial draft

Mark, Mehdi:

Attached is a first draft.

Malcolm Lancaster

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From: Crane, Mark (LI, Geelong)
Sent: Friday, 10 February 2006 2:29 PM
To: GA - Fish Diseases Laboratory
Cc: Mehdi Doroudi (mehdi.doroudi@dpi.vic.gov.au); Malcolm Lancaster (malcolm.lancaster@nre.vic.gov.au)
Subject: Abalone transmission trial

Dear All,

As discussed at our project meeting, we are working with Victoria on an abalone transmission trial associated with the recent disease outbreak in Western Victoria. Some information that we know about the disease (provided by Victoria) and the draft experimental plan is attached for information. Please send any comments to me.

Mark

Abalone pesvirus 060208.d(

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Herpes-like virus of abalone

The disease

Clinical Presentation: Significantly increased mortality. Moribund and dead abalone display enlarged mouths, some with protruded radula, foot becomes curled – often to the point that the sides meet.

Pathology: Histopathology restricted to nervous system – distinctive haemocytic infiltration of ganglia and nerves (ganglioneuritis with haemocytic infiltration).

Electron Microscopy: The viruses were present in necrotic and what appeared to be health nuclei of the ganglia. The viruses were icosahedral with electron dense cores. The diameter was 104nm +/-4 (n=17). The size of the naked viruses is characteristic of herpesviruses as is the nuclear location and varied nature of the electron dense cores. One particle was observed external to the nucleus.

The ultrastructure of the viruses was similar to that described by Chang et al (Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan), *Dis Aquat Org*, 63: 23-27, 2005.

Incubation Period: Epidemiological investigation suggests that clinical disease and mortality occur at around 10-14 days post-exposure.

Host Factors: Greenlip, hybrid and blacklip abalone are susceptible and 1-year-old stock (ca. 40mm) appear to be more severely affected than older stock. Young abalone (<10 mm) have not been affected.

Transmission trial

Location: C8

Aims

- To determine whether the disease can be reproduced experimentally
- To determine whether virus stored frozen at -80^{o}C is viable

Materials

Healthy abalone (1-year-old hybrids: female blacklip x male greenlip) will be provided by Great Southern Waters, Indented Head.

Infected material (live, diseased abalone; frozen infected material; ex Portland) will be supplied by PirVic.

Filtered seawater will be supplied by MAFRI, Queenscliff.

Aquarium facilities will be supplied by AAHL.

Experimental design

5 experimental groups:

1. Untreated (normal) controls: 1 tank with 10 abalone
2. Co-habitated abalone: 10 healthy abalone with 5 sick abalone (X2 tanks)
3. Abalone injected with frozen material: 10 abalone (X2 tanks)
4. Abalone injected with fresh infected material: 10 abalone (X2 tanks)
5. Abalone injected with medium only: 10 abalone (X2 tanks)

Calendar

Wed 8 Feb:	Flow-through rates checked; water tanker (McColl's) ordered
Thurs 9 Feb:	System ready to receive seawater Infected abalone (50) ordered through Mehdi
Fri 10 Feb:	Filtered (30 µm) seawater from MAFRI delivered
Mon 13 Feb:	Healthy abalone (100) from GSW (pm) – Mark to pick up
Tues 14 Feb:	Frozen, diseased abalone (ex Portland) from PirVic (am) Histology samples taken from healthy and sick abalone Fresh, diseased abalone (ex Portland) from PirVic (pm) Process inoculum Infect abalone

Wed 15 Feb: Turn on flow-through system

16 Feb-2 Mar: Check abalone daily for disease signs, record mortality, water quality measurements, and sample as appropriate

Sampling

When disease/mortality occurs, on a daily basis, collect samples for:

Histology
Electron microscopy
Virus isolation

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From: Partridge, Anne (LI, Geelong)
Sent: Wednesday, 15 February 2006 1:14 PM
To: Crane, Mark (LI, Geelong); Walker, Peter (LI, Geelong)
Subject: FW: [UNCLASSIFIED]AqCCEAD OOS 2006-03- response
Attachments: AqCCEAD OOS_2006-03_ Response_minutes.doc

Importance: High

Follow Up Flag: Follow up
Flag Status: Completed

Hi Mark/Peter,
Do you have any comments - if you have replied direct please copy me the response so that I can include in TRIM. If you have comments now I can include them in the response sheet.
Anne

Anne Partridge
PA to Dr Martyn Jeggo
Director
Australian Animal Health Laboratory (AAHL)
CSIRO Livestock Industries
PMB 24
GEELONG VIC 3220
Tel: 03 5227 5160
Fax: 03 5227 5250
email: Anne.Partridge@csiro.au
email: Martyn.Jeggo@csiro.au

-----Original Message-----
From: Alistair.Herfort@affa.gov.au [mailto:Alistair.Herfort@affa.gov.au]
Sent: Wednesday, 15 February 2006 11:43 AM
To: Hugh.Millar@dpi.vic.gov.au; bruce.christie@agric.nsw.gov.au; Jeggo, Martyn (LI, Geelong)
Cc: Ingo.Ernst@affa.gov.au; Karina.Scott@affa.gov.au
Subject: [UNCLASSIFIED]AqCCEAD OOS 2006-03- response
Importance: High

Dear Aq CCEAD Member

You will recall a copy of the Draft Minutes arising from the Aquatic CCEAD teleconference, convened Friday 3rd February 2006, to discuss implications of recent abalone mortalities in Victoria. Draft minutes were circulated by facsimile (8/2/06) for COMMENT (with supplementary attachments forwarded by fax later the same day), with responses requested by COB Tuesday 14th February.

If you have not already done so, could you **please respond** to AqCCEAD out-of-session item 2006/03 (using the response sheet attached here for convenience), **by facsimile** as soon as possible? If you have no comments, please respond with "No Comment".

Comments received will be incorporated into revised draft minutes and re-circulated for final endorsement by AqCCEAD.

PLEASE SEND RESPONSE SHEET BY FACSIMILE TO: AqCCEAD SECRETARIAT. on 02 6273 5237

If you require the draft Minutes to be re-sent to you, please let me know by reply email or telephone 02 6272 4009 as soon as possible.

Kind regards

Alistair Herfort
Aquatic Animal Health
Product Integrity, Animal and Plant Health
Australian Government Department of Agriculture, Fisheries and Forestry
GPO Box 858
Canberra ACT 2601
Ph: 02 6272 4009 Fax: 02 6272 3372
E-mail: alistair.herfort@daff.gov.au
Web page: http//:www.daff.gov.au/aquaticanimalhealth

AQUATIC CONSULTATIVE COMMITTEE ON EMERGENCY ANIMAL DISEASES (Aq CCEAD)

OUT OF SESSION PAPER – RESPONSE SHEET

Aq CCEAD OOS 2006/03

PAPER AUTHOR: Aq CCEAD Secretariat

SUBJECT: Aq CCEAD teleconference/meeting 1/2006 – Comment on draft minutes

FOR RESPONSE BY: 14 February 2006

DATE OF REPLY:

NAME AND JURISDICTION:

COMMENTS.

Signature of AqCCEAD Member

From: Crane, Mark (LI, Geelong)
Sent: Friday, 17 February 2006 3:03 PM
To: Williams, Nette (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: McColl, Ken (LI, Geelong)
Subject: RE: Abs and zebra

Follow Up Flag: Follow up
Flag Status: Completed

We cannot decide this until some analysis is undertaken - histo and EM.

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-----Original Message-----
From: Williams, Nette (LI, Geelong)
Sent: Friday, 17 February 2006 15:01
To: Corbeil, Serge (LI, Geelong)
Cc: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong)
Subject: RE: Abs and zebra

I am doing the abs Saturday and Sunday morning so I will email when I have been and let the next person know what I found out.

I will sample for Histo and PCR this afternoon when I go over

Won't feed fry on Sunday

Mmm when do we decide if they died of disease or was it human error/intervention?

Nette

-----Original Message-----
From: Corbeil, Serge (LI, Geelong)
Sent: Friday, 17 February 2006 1:00 PM
To: Williams, Nette (LI, Geelong)
Cc: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong)
Subject: Abs and zebra

Hi Nette,

Ken and I went to C8 this morning and found that the abs injected with the fresh virus were all dead or dying, yes! (tanks 3 and 4) and some abs injected with the frozen virus have started dying (tanks 7 and 8). The control abs (tanks 5-6-9) are fine which is great news (for us researchers). That is going to be an interesting paper. The co-habitation abs seem fine so far, they might start kiking the tank (bucket) within 2 weeks though. The sick ones in the baskets were dead, stinking and decaying therefore were a health hazard to the healthy ones :0) so I discarded them.

We took plenty of samples for histo/EM/PCR/virus isolation so from now on we will not need samples from the groups injected with the virus (but we will take some from the co-hab groups when they get sick.

Would you mind (as we forgot this morning) taking two abs from control DMEM and getting samples for histo (jars are made up and available) and for PCR (ganglion).

Jawahar informed me today that the baby zebrafish must not be overfed so you don't have to feed them Sunday.

Thanks,

Serge

From: Corbeil, Serge (LI, Geelong)
Sent: Saturday, 18 February 2006 1:16 PM
To: Williams, Nette (LI, Geelong)
Cc: McColl, Ken (LI, Geelong); Crane, Mark (LI, Geelong)
Subject: RE: Abs Saturday morning

Follow Up Flag: Follow up
Flag Status: Completed

Hi Nette,

Thanks for all this.
Sorry I forgot to mention that Nick asked me to put the EM fixative in the other fridge as he needed the space (I told Ken when I bumped into him but I did not manage to send an email and then forgot.

With regards to virus extraction/purification the idea is to freeze the virus and have the best chances of it staying alive. There is no point in trying to homogenise the ganglion as they are too small, I think putting the ganglions in DMEM 10% FCS and freezing them at -80 oC is the best way to preserve the virus.

Talk to you later,

Serge

-----Original Message-----
From: Williams, Nette (LI, Geelong)
Sent: Saturday, 18 February 2006 09:50
To: Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: McColl, Ken (LI, Geelong)
Subject: RE: Abs Saturday morning

Saturday morning

Controls are fine and had water change in square tank

Tank 1 Cohabitation
3 dead and 3 moribund (sampled for PCR, EM, Histo and virus isolation)

Tank 2 cohabitation
7 dead (only took histo on one animal that had white protrusions from it's foot, may not be any use)

Tank 4 - fresh inoculated

All are fine

I made up *Virkon* to spray the lunch box and samples before removing from the room

In the lab I prepared some EMEM with 10%FBS to store virus isolation samples in prior to freezing – it is in the Westinghouse fridge on Serges shelf

Stored the virus isolation samples with media as above, in freezer GA640 (near safety shower) on Serges shelf in ziplock bag

PCR sample yesterday and today is also in that freezer in ziplock bag

EM samples in lab 110 fridge on the bottom shelf in a rack where the fixative is - HL6258 (I had to "find" the fixative to do this!!!)

I also bought out the dead animals and put in the freezer as they are going to really stink, as the bin does, by Monday morning

In Mark's email below he says to take tissues for virus extraction- does he mean like we did to prepare the inoculum or does he mean virus isolation (for me to try and grow the virus) ?

If that is the case when do we do that as the animals may all die soon and we will end up with nothing.....

I will check my email before going in Sunday morning

Nette

-----Original Message-----
From: Crane, Mark (LI, Geelong)
Sent: Friday, 17 February 2006 1:14 PM
To: Corbeil, Serge (LI, Geelong); Williams, Nette (LI, Geelong)
Cc: McColl, Ken (LI, Geelong)
Subject: RE: Abs and zebra

Serge,

As we discussed yesterday, it will be worthwhile to take tissues for virus extraction (clarified tissue homogenate) and storage at -80 degrees C with 10% serum - this may be a more efficient way of storing viable virus.

Mark

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From: siobhan.connell@dpi.vic.gov.au on behalf of Mehdi.Doroudi@dpi.vic.gov.au
Sent: Monday, 20 February 2006 4:15 PM
To: McColl, Ken (LI, Geelong)
Cc: Crane, Mark (LI, Geelong); Malcolm.Lancaster@dpi.vic.gov.au
Subject: Re: Abalone expts

Follow Up Flag: Follow up
Flag Status: Completed

Categories: NQS

Thanks Ken, very interesting results. I have been expecting that because of the extent of the incident. It is good to know that the virus was viable in from material. Please keep me posted with the results of processing samples. Perhaps, someone should start writing the first draft. Do you want to talk with Malcolm about that. I hope that one of you guys will have the time to write it up.

Regards
Mehdi Doroudi - DVM, PhD
Research Director
Marine & Freshwater Systems - PIRVic
Department of Primary Industries

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Email: mehdi.doroudi@dpi.vic.gov.au
2A Bellarine Hwy, Queenscliff VIC 3225 Australia
PO Box 114, Queenscliff VIC 3225 Australia

Kenneth.McColl@csiro.au
20/02/2006 12:19 PM

To: Mehdi.Doroudi@dpi.vic.gov.au
cc:
Subject: Abalone expts

Hi Mehdi,

Just to keep you informed about the abalone expts, here are some preliminary results.

By the end of this w/e (just completed, ie, about 4-5 dpi), we've got the following results:

Negative controls (no treatment): no mortalities
Negative controls (2 tanks; about 16 abalone in each; all abs

inoculated I/M with tissue-culture media): no mortalities

Treatment group 1 (2 tanks; about 16 in each tank; all inoculated with fresh material from Portland abalone): all dead or moribund

Treatment group 2 (2 tanks; about 16 in each tank; all inoculated with frozen material from an earlier outbreak): all dead or moribund.

Treatment group 3 (2 tanks; about 16 in each tank; healthy abs co-habitated with 5 moribund abs from Portand): only one healthy ab remaining in each tank; all others dead or moribund

Tissues have been collected for histo, E/M, PCR and attempted virus isolation.

Still a lot of processing of samples to be done, but it certainly looks like something is happening!

Best wishes,

Ken

PS. Mark is trying to call Malcolm Lancaster at the moment to provide him with these results too.

Dr Ken McColl BVSc PhD
CSIRO-AAHL
PO Bag 24
Geelong Vic 3220
Australia

Telephone: (03) 5227 5104
E-mail: kenneth.mccoll@csiro.au

17

From: Crane, Mark (LI, Geelong)
Sent: Monday, 27 February 2006 11:07 AM
To: McColl, Ken (LI, Geelong); 'Malcolm.Lancaster@dpi.vic.gov.au'; Corbeil, Serge (LI, Geelong)
Cc: 'Mehdi.Doroudi@dpi.vic.gov.au'
Subject: RE: Abalone #2
Attachments: Abalone 2 060227 Rev Protocol.pdf

Follow Up Flag: Follow up
Flag Status: Completed

Thanks, Ken, for pointing out my cutting and pasting error. Revised protocol is attached.

MARK CRANE Ph.D.
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Geelong Vic 3220

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International Fax: +61 3 52 275555
email: mark.crane@csiro.au

-----Original Message-----
From: McColl, Ken (LI, Geelong)
Sent: Monday, 27 February 2006 10:40
To: Crane, Mark (LI, Geelong); 'Malcolm.Lancaster@dpi.vic.gov.au'; Corbeil, Serge (LI, Geelong)
Cc: 'Mehdi.Doroudi@dpi.vic.gov.au'
Subject: RE: Abalone #2

Sounds good to me Mark. I agree about the virus - some herpes are very tough; others we don't know about. Until we do, we should assume that the 4 hr trip is not good for virus viability.

I presume you mean 800 mL to be placed in the tanks in the last two steps in D5 (as per your diagram).

Ken

-----Original Message-----
From: Crane, Mark (LI, Geelong)
Sent: Monday, 27 February 2006 10:16 AM
To: McColl, Ken (LI, Geelong); 'Malcolm.Lancaster@dpi.vic.gov.au'; Corbeil, Serge (LI, Geelong)
Cc: 'Mehdi.Doroudi@dpi.vic.gov.au'
Subject: RE: Abalone #2

I am concerned that the water has to travel 4 hours from Portland to Geelong with the virus deteriorating all the time. My suggestion is that we generate inoculum at AAHL with diseased abalone from Portland as in the previous experiment (see attached protocol). In this way the virus is "fresh". Please let me know what you think.

This experiment will cost more than $20,000.00, depending how long it runs for. The breakdown is as follows:

LAF room C8: 20 days @ $80.00 per day = $1600.00

Salaries: $4000
Consumables: $400
Indirect costs:$17000 (biosecurity, corporate overheads etc)

Clearly, since this is a joint collaboration, we do not expect to obtain full cost recovery but AAHL would expect to get at least 50% cost recovery.

Mark

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Australian Animal Health Laboratory
CSIRO Livestock Industries
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email: mark.crane@csiro.au

-----Original Message-----
From: McColl, Ken (LI, Geelong)
Sent: Monday, 27 February 2006 09:35
To: 'Malcolm.Lancaster@dpi.vic.gov.au'
Cc: Mehdi.Doroudi@dpi.vic.gov.au; Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong)
Subject: RE:

Hi Malcolm,

I've made a couple of suggested changes, but nothing major. I think your second aim, as stated, may be a bit ambitious, and I suggest we just state what we're actually trying to do, ie, look at the effect of dilution of sea-water as a form of control.

Ken

-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Friday, 24 February 2006 2:47 PM
To: Crane, Mark (LI, Geelong)
Cc: McColl, Ken (LI, Geelong); Mehdi.Doroudi@dpi.vic.gov.au
Subject:

Hi Mark:

Attached is a draft protocol for further exploration of the epidemiology of the herpes-like virus of abalone.
Could you
(a) comment on the suitability of this protocol
(b) provide an approximate costing?

I have added an additional cohabitation trial - because I wasn't sure whether there was the possibility of direct contact between affected abalone and naive abalone in the trial just completed.

Thanks,
Malcolm Lancaster

ABALONE #2

D0 (Thurs): Set up C8

D1 (Fri): Put healthy (ex Great Southern Waters) experimental animals in aquaria (T1-9). No feeding.

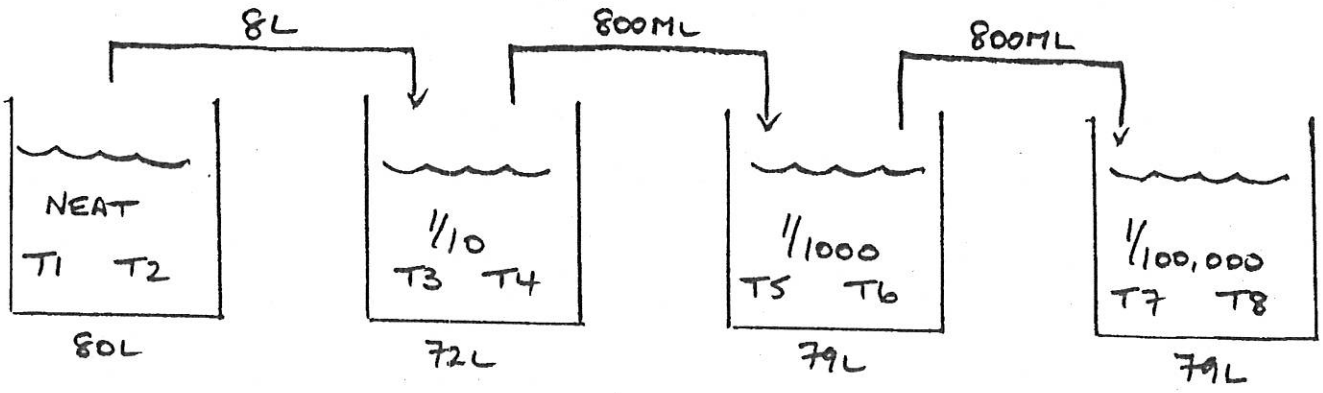
D4 (Mon): Clean tanks. Tank 9 will house untreated, negative controls.

D4 (Mon): Place diseased animals in baskets in Tanks 1 and 2 (positive controls). Static tanks for 24 hours. In addition, these will provide the inoculum for the other experimental infections.

D5 (Tues): Remove 8L each from T3 and T4. Take 8L each from T1 and T2, mix (total of 16L) and place 8L in each of T3 and T4 (1/10). Static tanks for 24 hours.

Remove 800mL each from T5 and T6. Take 800mL each from T3 and T4, mix (total of 1.6L) and place 800mL in each of T5 and T6 (1/1000). Static tanks for 24 hours.

Remove 800mL each from T7 and T8. Take 800mL each from T5 and T6, mix (total of 1.6L) and place 800mL in each of T7 and T8 (1/100 000). Static tanks for 24 hours.



D6 (Wed): Put all tanks on flow-through. No feeding.

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From: McColl, Ken (LI, Geelong)
Sent: Monday, 27 February 2006 5:34 PM
To: Crane, Mark (LI, Geelong); 'Malcolm.Lancaster@dpi.vic.gov.au'; Corbeil, Serge (LI, Geelong)
Cc: 'Mehdi.Doroudi@dpi.vic.gov.au'
Subject: RE: abalone virus trial #2

Follow Up Flag: Follow up
Flag Status: Completed

What Mark says sounds reasonable to me, as does the request from Malcolm to look at the water coming out of CSF. A few comments and observations:

1. I don't think the absence of a flow-through system in the second LAF room would matter too much (unless the experiment went on for 10-20 days). We would need to do a 1/4 or 1/2 change of water in these tanks every few days, but that would have very little effect on the titre of virus in the tanks (eg, each 1/2 change in the water results in a decrease in the virus titre of 0.3 logs per unit volume - hardly significant if the original titre is, say, 3 or 4 (or more) logs per unit volume, AND the expt is relatively short-term).

2. My gut feeling would be that, so long as the water from Portland is not transported at 37C, then the 4-hour trip is probably not significant. If the air-conditioner in a station wagon was set as cold as possible, then the water temperature may not even change too much during the trip. All of the work done at AAHL so far has been at, what, 15C? We could probably maintain water at about that temp during the trip reasonably easily.

3. I agree with Mark that starting with the 1:10 dilution of CSF water is not ideal. However, based on what we saw in the first trial, you'd have to believe that a 1:10 dilution will be infectious.

I'll think about it more overnight!

Ken

-----Original Message-----
From: Crane, Mark (LI, Geelong)
Sent: Monday, 27 February 2006 4:45 PM
To: 'Malcolm.Lancaster@dpi.vic.gov.au'; McColl, Ken (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au
Subject: RE: abalone virus trial #2

Anything is possible, Malcolm!

I think that if we are going to do this, then we would need to set up a second LAF room. For instance, if the CSF water turned out to be infectious then we would want a titration done. So we may as well do that now rather than in a further (third) experiment. However, we only have one room set up for flow-through. We have to decide on how to manage the exposure. Do we want flow-through, or static, or static with daily partial water changes - in both rooms or just in one of the rooms? The experiment is likely to go for a longer time period - more water will be used. That is why I put a 20-day time period on it.

If CSF water (80 Litres - we would need to have duplicates) is transported to AAHL (on ice?), it will not be easy to get it into the LAF (10 x 10 litre buckets?). One option is to start the titration at 1/10 - we would only need about 16 litres (8 litres in each of two 80 litre tanks) and then we could do the 1/1000 and 1/100000 dilutions. However, if we start at 1/10 and the water is not infectious would be because of the 4 hour travel time or because of the 1/10 dilution?

Thoughts please!

Costing:

FYI, I've just received the bill for water transportation: $350.00.

Extra room rent would be $1600
Extra overheads
Extra water (2 rooms for 20 days = 30 000L) = $1000 for 3 deliveries of 10,000L each.

A two room experiment will cost $25,000 (instead of $20,000 for one room) - our labour would not be increased too much. AAHL management would probably want an external contribution of $15,000. AAHL would provide $10,000 in-kind.

Cheers

Mark

MARK CRANE Ph.D.
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CSIRO Livestock Industries
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-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Monday, 27 February 2006 14:34
To: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au
Subject: abalone virus trial #2

Hi Mark:

Thanks for all the comments.
It would be nice to put some naive abalone into the seawater that actually emerges from CSF, as this is the best approximation to what the wild abalone could be exposed to. Given that diseased animals have to be transported from CSF, is it possible to set up another tank in C8 and fill it with 80 litres of 4hour old water also transported from CSF?

Regards,
Malcolm

19

From: McColl, Ken (LI, Geelong)
Sent: Thursday, 2 March 2006 12:49 PM
To: 'Malcolm.Lancaster@dpi.vic.gov.au'; Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au
Subject: Abalone virus transmission trial

Follow Up Flag: Follow up
Flag Status: Completed

Hi guys,

We didn't get the histo sections from the transmission trial until yesterday, and I've just finished reading all the slides.

The severe cases are easy to identify, but the most acute cases can sometimes be troublesome, and it would be good to sit with Malcolm over a double-header at some time to look at some cases together. So, the results:

06-0441 (healthy abalone - arrived at AAHL Mon 13.2.06)

In general, there appear to be no lesions in these abalone, but, in at least one case, there appear to be occasional foci of pyknotic nuclei in the neurilemma around the ganglia.
In another case, there is a moderately severe inflammatory lesion in the submucosa of the foot, but, in this case, the epithelium is missing (presumably because of the over-zealous use of the paint-scraper!), and therefore I believe the lesion is unrelated to the herpesvirus.

06-0442 (affected abalone from Portland - arrived at AAHL Tues 14.2.06. These were our source of freshly-prepared virus)

In general there is severe, diffuse necrosis of the ganglia with oedema and infiltration of haemocytes. Lesions are present in the ganglia, nerve tracts and in the lamina propria and submucosa of various sites, eg, oesophagus, other parts of the GI tract, and the gills.

EXPERIMENTAL ANIMALS

06-0520 (negative control abalone inoculated with tiss. culture medium)

No visible lesions in these samples

06-0521 (healthy abalone that were co-habitated with known infected abalone)

Lesions in the ganglia, nerve tracts, submucosal and LP sites as described above. Lesions ranged from 'severe and diffuse' to 'acute and multifocal' (suggesting that, as would be expected, the healthy abalone were infected at different times after exposure to the infected abalone).

06-0522 (healthy abalone inoculated with freshly-prepared infectious samples)

Again, lesions ranging from 'peracute' to 'acute and diffuse'.

06-523 (healthy abalone inoculated with frozen infectious samples)

Same range of lesions.

Based on the apparent "age" of some lesions, it is tempting to speculate on the pathogenesis of this disease, but such speculation is probably not really justified until we look at many more cases (that have been infected via a natural route of infection, such as in our co-habitation experiements).

Ken

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20

From: Malcolm.Lancaster@dpi.vic.gov.au
Sent: Monday, 6 March 2006 9:59 AM
To: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au; bronwyn.murdoch@dpi.vic.gov.au
Subject: abalone virus transmission trial 2
Attachments: Abalone virus trial 2 Mar06 short.doc

Follow Up Flag: Follow up
Flag Status: Completed

Hi guys:

Attached is a draft for your comments. I am aiming to get a consensus version to Hugh Millar CVO Victoria by Wednesday.

Thanks,
Malcolm

Abalone virus wild fisheries risk assessment

Background

Deaths preceded by paralysis have occurred in greenlip (*Haliotis laevigata*), blacklip (*Haliotis rubra*) and hybrid abalone on 3 farms. Up to 90% mortality has occurred in individual tanks.
Histologically, there is ganglioneuritis in affected abalone from all 3 farms. A herpes-like virus has been seen electron microscopically in the pleuropedal ganglion of an affected abalone from farm 1.
Herpes viruses are relatively fragile, and may require close contact for transmission.
Experimentally, disease has been transmitted to naïve abalone by inoculation of infected material from affected abalone, or by cohabitation with affected abalone.
Disease has apparently been eliminated from farms 2 and 3, but is still occurring on farm 1.
The source of the virus has not been established. Wild brood-stock from a number of Australian sites were introduced to farm 1 prior to the outbreak. A similar virus has been reported in Taiwan.

Concerns

Farm 1 is an open system with water in contact with affected abalone re-entering the Southern Ocean. As wild abalone are present within a hundred metres or so of the farm 1 outlet then there is a risk that virus from affected farmed abalone could infect nearby wild abalone, with subsequent spread east and west along the Victorian coast (and interstate).

Proposal

Aims

(1) to establish if the virus can transmit through the water column
(2) to determine if dilution of contaminated water can be used as a means of disease control

Methods

Negative control animals:
Group 1: 30 abalone maintained for 2 weeks in seawater collected well away from any abalone farm ("normal" seawater from an unaffected site, eg, Queenscliff).

Positive control animals
Group 2: 30 abalone sharing the same water as affected abalone, but with no possibility of direct contact (double-caging).

Test animals
(1) Static water from affected abalone held at AAHL
Group 3: 30 abalone exposed to seawater collected from affected tank at AAHL diluted **1:10**.
Group 4: 30 abalone exposed to seawater collected from affected tank at AAHL diluted **1:1000**.
Group 5: 30 abalone exposed to seawater collected from affected tank at AAHL diluted **1:100,000**.

(2) Water from affected farm 1
Group 6: 30 abalone exposed to seawater collected from affected farm 1 diluted **1:10**.
Group 7: 30 abalone exposed to seawater collected from affected farm 1 diluted **1:1000**.
Group 8: 30 abalone exposed to seawater collected from affected farm 1 diluted **1:100,000**.

Costs

A two room experiment will cost $25,000 (instead of $20,000 for one room) - our labour would not be increased too much.

LAF room C8: 20 days @ $80.00 per day = $1600.00
Salaries: $4000
Consumables: $400
Indirect costs:$17000 (biosecurity, corporate overheads etc)

Extra room rent would be $1600
Extra overheads
Extra water (2 rooms for 20 days = 30 000L) = $1000 for 3 deliveries of 10,000L each.

Clearly, since this is a joint collaboration, we do not expect to obtain full cost recovery but AAHL would expect to get at least 50% cost recovery.
AAHL management would probably want an external contribution of $15,000.
AAHL would provide $10,000 in-kind.

From: Corbeil, Serge (LI, Geelong)
Sent: Tuesday, 7 March 2006 10:48 AM
To: 'Malcolm.Lancaster@dpi.vic.gov.au'
Cc: 'bronwyn.murdoch@dpi.vic.gov.au'; McColl, Ken (LI, Geelong); Crane, Mark (LI, Geelong); 'Mehdi.Doroudi@dpi.vic.gov.au'
Subject: RE: abalone virus transmission trial 2

Follow Up Flag: Follow up
Flag Status: Completed

Hi Malcom,

Yes of course we should reproduce the previous results with the caged animals (sick abs in the basket above the healthy ones) and use that water
after 24 hours (enough time for the sick abs to shed the virus) for the dilution experiment. We would keep the "caged animal tank" going to make sure the healthy abs die.

Cheers,

Serge

-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Monday, 6 March 2006 17:15
To: Corbeil, Serge (LI, Geelong)
Cc: bronwyn.murdoch@dpi.vic.gov.au; McColl, Ken (LI, Geelong); Crane, Mark (LI, Geelong); Mehdi.Doroudi@dpi.vic.gov.au
Subject: RE: abalone virus transmission trial 2

Thanks Serge.
Mark also told me that there was no direct contact - and your comment re timing of morbidity and mortality is a good one.
I left the double-cage trial in to replicate this result - and to have one group at least in the second trial that came down with the disease! (or is that overkill?)

Malcolm

Serge.Corbeil@csiro.au

06/03/2006 11:03 AM

To: Malcolm.Lancaster@dpi.vic.gov.au, Mark.Crane@csiro.au, Kenneth.McColl@csiro.au
cc: Mehdi.Doroudi@dpi.vic.gov.au, bronwyn.murdoch@dpi.vic.gov.au
Subject: RE: abalone virus transmission trial 2

Hi Malcom,

Only two comments on the methodology;

-Aim 1: we already have demonstrated that the virus can be transmitted through the water column. Despite the fact that some abalone climbed on the wall of the tanks they did not touch the basket which stands slightly away from the side. Furthermore, most abalone stayed at the bottom clumped at the same place during the course of the experiment (in fact I noticed that after 24 hours the abalone that climbed on the wall had chosen a spot and stayed there) and all the abalone from the co-habitation trial exhibited morbidity and mortality pretty much at the same time frame suggesting that the virus spreads through the water.

-It would be more accurate to write 15 abalone per group in duplicate.

Cheers,

Serge
-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Monday, 6 March 2006 09:59
To: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong); Corbeil, Serge (LI, Geelong)
Cc: Mehdi.Doroudi@dpi.vic.gov.au; bronwyn.murdoch@dpi.vic.gov.au
Subject: abalone virus transmission trial 2

Hi guys:

Attached is a draft for your comments. I am aiming to get a consensus version to Hugh Millar CVO Victoria by Wednesday.

Thanks,
Malcolm

22

From: Malcolm.Lancaster@dpi.vic.gov.au
Sent: Friday, 17 March 2006 11:14 AM
To: Crane, Mark (LI, Geelong)
Subject: RE: Abalone trials

Follow Up Flag: Follow up
Flag Status: Completed

Mark:
It was Hugh Millar who wanted a slightly more detailed proposal, to approach DAFF/Bob Biddle for some funding in the short term (he'd received some encouragement from them, but more detail was required).
Malcolm

Mark.Crane@csiro.au

17/03/2006 10:19 AM

To: Malcolm.Lancaster@dpi.vic.gov.au
cc: Kenneth.McColl@csiro.au, Lynette.Williams@csiro.au, Serge.Corbeil@csiro.au, mehdi.doroudi@dpi.vic.gov.au
Subject: RE: Abalone trials

I had a chat with Mehdi yesterday and he clarified a few points. He will organise getting water and sick abalone from Portland as before. He will discuss how to get the water to Geelong in the best possible way (e.g. 20 litre drums - we will need a trolley and access to the LAF via the animal entry) with his staff and then let us know when they are ready to transport. With respect to funding this current work, Mehdi said that Victoria will take care of it one way or the other and we do not need to do anything.

With respect to timing - we need to wait for MAFRI to get the water from the affected farm.

Mark

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-----Original Message-----
From: Malcolm.Lancaster@dpi.vic.gov.au [mailto:Malcolm.Lancaster@dpi.vic.gov.au]
Sent: Thursday, 16 March 2006 13:29
To: Crane, Mark (LI, Geelong)
Cc: McColl, Ken (LI, Geelong); Williams, Nette (LI, Geelong); Walker, Peter (LI, Geelong); Corbeil, Serge (LI, Geelong)
Subject: RE: Abalone trials

I have spoken to Tim Rudge at CSF, and given him a summary of the results from abalone 1. He is now expecting to be approached for further sick abs and 200 litres of water from the pond/lagoon (he has a pump that could be used), and is aware of the aims of abalone 2.

Tim is very keen to investigate the effects of temperature on the epidemiology of the disease. I indicated that such work would have to be done at AAHL, but that his company as the major beneficiary would have to be the major funder. If you are interested, you could consider what it might cost.

Cheers,
Malcolm

Mark.Crane@csiro.au

16/03/2006 11:58 AM

To: Kenneth.McColl@csiro.au, Serge.Corbeil@csiro.au
cc: Peter.Walker@csiro.au, Lynette.Williams@csiro.au, malcolm.lancaster@dpi.vic.gov.au
Subject: RE: Abalone trials

Malcolm just called to give us the go-ahead for Abalone 2. As Ken mentioned, as far as Victoria is concerned the experiment needs to include undiluted water from farm 1. This means that we will need to get 150-200 litres of water from this farm. Mehdi is visiting AAHL to meet with Peter and myself today - afterwards we should talk with Mehdi about the experiment and especially about getting water from farm 1. How we get this water into the LAF is another issue (presumable through the Animal Entry). We will need to talk with Don Carlson about this.

Malcolm said that we would get the requested $15,000 funding for the experiment one way or the other but, whichever way, we were guaranteed it. Mehdi has spoken with DAFF and FRDC (need to talk with Mehdi about this too) and there is the possibility of getting some immediate funds from these organisations to conduct this work - including the PCR work commenced by Serge. We will need to develop a proposal for DAFF/FRDC - I will contact Eva and FRDC to find out exactly what is needed. I know that the Executive Director of FRDC (Patrick Hone) does have discretionary authority to disburse funds upto $20,000 for small projects. Clearly, Victoria would prefer to have DAFF/FRDC fund the work but they will put up the money if necessary.

I will also talk with Steve Rodis at Great Southern Waters (Indented Head) about getting some more host abs. Malcolm said that Hugh Millar (CVO, Victoria) was comfortable with us disclosing the results to GSW since they were providing the animals. Malcolm will keep the affected farm at Portland informed about results and on-going work.

Once we have spoken with Mehdi and I have spoken with Steve Rodis (and Eva and FRDC) we will have a better idea of when we can commence this experiement - hopefully next week. We need to re-visit the protocol and make adjustments to include farm water groups. Perhaps we can meet tomorrow morning to discuss.

Mark

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email: mark.crane@csiro.au

-----Original Message-----
From: McColl, Ken (LI, Geelong)
Sent: Tuesday, 14 March 2006 15:18
To: Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong)
Subject: Abalone trials

Mark and Serge,

I've already mentioned this to Mark, but thought I'd better put it in writing (and also inform Serge).

When Malcolm Lancaster was here on Friday morning, we chatted about the future abalone work, and Malcolm said that two issues arose when he discussed the proposal with his colleagues at PirVIC:

1. They'd like to test, directly, the water from Farm 1 at Portland on susceptible abalone (which means transporting an appropriate amount of water to AAHL) - *we could probably do this just by bringing a tank of water from Portland in the back of a station wagon.*

2. They'd like an experimental set-up that absolutely guaranteed no contact between infected abalone and susceptible ones (in a co-habitation experiment) - *I suggested that we simply suspend the colander (containing the infected abalone) over the centre of the tank, rather than having it attached close to the side of the tank).*

Malcolm didn't say anything about money for the project, but I guess if they're talking about the details of the experiment, then they must be fairly committed to the idea.

Ken

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DOCUMENT 23

EXEMPT IN FULL – s 47B(a)

DELETION s22

-----Original Message-----
From: McColl, Ken (LI, Geelong)
Sent: Wednesday, 29 March 2006 15:08
To: Corbeil, Serge (LI, Geelong); Crane, Mark (LI, Geelong); Williams, Nette (LI, Geelong)
Subject: RE: Abalone expt

Serge,

We still have two tanks at 1:100,000 dilution to address the aim that you've mentioned.

What I'm talking about is changing the third tank at that dilution for the sequential study. Anyway, Nette says there is a discussion at 9 am tomorrow, so let's leave the discussion until then.

Ken

-----Original Message-----
From: Corbeil, Serge (LI, Geelong)
Sent: Wednesday, 29 March 2006 3:00 PM
To: McColl, Ken (LI, Geelong); Crane, Mark (LI, Geelong); Williams, Nette (LI, Geelong)
Subject: RE: Abalone expt

Ken,

I think the point of the experiment is to find out what dilution can "safely" be applied to the source of infection and related that to the field situation (assuming that it is possible). Therefore the 1:100 000 dilution is important as if the "first ab" get infected it will show that a similar scenario can occur in the field. The fact that it will amplify the virus is a normal process that also what occurs in the field.

Serge

-----Original Message-----
From: McColl, Ken (LI, Geelong)
Sent: Wednesday, 29 March 2006 14:51
To: Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong); Williams, Nette (LI, Geelong)
Subject: Abalone expt

Hi guys,

As I squirted 0.8 mL of farm-water into the 80 L tanks in C7, it seemed to me that perhaps the 0.001% dilution is not the best dilution for our sequential study. We might be better off making the third tank in this series another 10% or 0.1% dilution for our sequential studies.

At 0.001%, the likelihood of abs getting infected is probably low, and then, if one DOES become infected, then it will probably pump out huge amounts of virus, and everyone will quickly go down with disease. So the sequential study may reveal very little.

If we make it a 0.1% or especially a 10% tank, then it's more likely that all abs will become infected at about the same time (but at a lower titer than those in the undiluted farm water), resulting in sequential sampling generating more interesting results. We've got plenty of farm water left over, so we could easily still make the change.

Any thoughts?

Ken

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Robinson, Karen (Legal, Campbell)

From: Corbeil, Serge (LI, Geelong)
Sent: Friday, 31 March 2006 10:22 AM
To: Crane, Mark (LI, Geelong)
Subject: RE: Finalised? protocol

Follow Up Flag: Follow up
Flag Status: Completed

Actually, the purpose was not to sample them (as this experiment was done in February) but to confirm that the new abalone were susceptible to the virus and also to give us an idea of how long it takes for the virus to be shed and kill the healthy ones at the bottom. That way we will be able to roughly estimate the timeline of the current co-hab experiment (with farm sick abs).

Serge

-----Original Message-----
From: Crane, Mark (LI, Geelong)
Sent: Friday, 31 March 2006 07:56
To: Corbeil, Serge (LI, Geelong)
Subject: RE: Finalised? protocol

This means we have very few (4 inoculated abalone in each of two tanks) real positive control abalone - these need to be sampled but are also being used to infect co-habitants? Timing of the sampling is going to be critical

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-----Original Message-----
From: Corbeil, Serge (LI, Geelong)
Sent: Friday, 31 March 2006 07:32
To: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong); Williams, Nette (LI, Geelong)
Subject: RE: Finalised? protocol

Dear all,

There has been some confusion over the extra experimental group in C6 probably due to the original mislabelling, in fact there are only 6 groups and not 7. I have made the corrections in red.

Serge

<< File: ABALONE 2 31-03-06.doc >>

-----Original Message-----
From: Crane, Mark (LI, Geelong)
Sent: Thursday, 30 March 2006 15:01
To: Corbeil, Serge (LI, Geelong); McColl, Ken (LI, Geelong); Williams, Nette (LI, Geelong)
Subject: Finalised? protocol

When Serge and I checked on the expt today, the Abalone looked fine - no mortalities. Water temp = 18 C. Water had only small amount of faeces.

Two extra record sheets will be needed in C8 - there were only 2 and there are 4 tanks.

As discussed, we created another experimental group - Group 5 in C7 (1% farm seawater). It is tank #1 with its own net and siphon hanging next to it. It ended up with 17 abalone because C8 had less than its full complement.

Latest up-date of protocol is attached - to include the extra experimental group in C6.

Mark
<< File: ABALONE 2 060330.doc >>

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ABALONE #2

Three LAF rooms are required:

Uninfected Room (C8)

Group #	Treatment	# Tanks
1	No treatment	2 (10 abalone ea.)
2	Sham (EMEM + 10% FBS) inoculated (30 March)	2 (10 abalone ea.)

Experimental Room (C7)

Group #	Treatment	# Tanks
1	100% farm water for 48 hours	2 (12 abalone ea.)
2	10% farm water for 48 hours	2 (1/10 farm water)
3	0.1% farm water for 48 hours	2: 80 mL farm water
4	0.001% farm water for 48 hours	2: 0.8 mL farm water
5	1% farm water for 48 hours – **sequential study**	1: 800 mL farm water (17 abalone) #13

Positive Controls Room (C6)

Group #	Treatment	# Tanks
1	Co-habitation (no contact) with 4 GSW abalone inoculated with frozen sample from Portland (Trial 1)	2 (10 abalone at bottom of tank ea.)
2	Co-habitation (no contact) with sick abalone from Portland	2 (replace water at 50% mortality)
3	100% water from group #2 for 48 hours*	2 (70 Litres ea.)
4	10% water from group #2 for 48 hours*	2 (ca. 8 litres ea.)
5	0.1% water from group #2 for 48 hours*	2 (ca. 80 mL ea.)
6	0.001% water from group #2 for 48 hours*	2 (0.8 mL ea.)

Total of 25 tanks with 10-17 abalone each > 300 abalone

Diary

D -2 (Mon): Put healthy (ex Great Southern Waters) experimental animals in aquaria. No feeding.

D 0 (Wed): Place diseased animals in baskets in co-habitation tanks (static for 24 hours).
Place farm water in tanks.
Prepare inoculum for the other experimental infections.
Inoculate positive controls.

D 1 (Thurs): Take untreated control samples (x2) from C8 for histology.
Sham inoculate (EMEM + 10% FBS) uninfected control group.

D 2 (Fri): 50% water change today and every second day onwards

*Replace clean water from positive control groups 3-6 with infectious water from positive control group #2 as per schedule – when approximately 50% mortality occurs.

Sampling

Samples (x2) for histopathology and electron microscopy need to be taken as soon as abalones start dying. All moribund abalone, when they occur, should be taken for histology.

Sequential study (C7 Group 5)

Samples will be taken in an attempt to obtain examples of early lesions. Two abalones will be sampled for histology at 2, 4, 6 and 8 days post-exposure to 1% farm water.

26

From: Crane, Mark (LI, Geelong)
Sent: Tuesday, 18 April 2006 1:15 PM
To:
Cc: 'Mehdi. Doroudi'
Subject: RE: On farm disease trials

DELETION

Follow Up Flag: Follow up
Flag Status: Completed

Just got back from meetings in Canberra and the Easter break. Trying to get through my emails quickly. While in Canberra I had a good discussion with Ann and she is happy for me to work with Malcolm on the proposal etc.

The attachment you mentioned in your email was not attached - could you please resend?

I have not heard of a recent fish kill in Tasmania due to a herpesvirus - I would suspect that Tasmanaian officials would have contacted us but I have not heard anything.

We were not involved with the sperm whale incident.

One thing about herpesviruses is that they tend to be species-specific. It would be rare for a finfish virus to infect shellfish, for instance. If you remember the pilchard herspesvirus incidents in 1995 and 1998 - no other fish species, only pilchards, were affected.

Mark

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email: mark.crane@csiro.au

From: Crane, Mark (LI, Geelong)
Sent: Wednesday, 19 April 2006 2:55 PM
To: 'annflem@bigpond.net.au'; Malcolm.Lancaster@dpi.vic.gov.au; mg@som-abalone.com.au
Subject: RE: On farm infection trial

Follow Up Flag: Follow up
Flag Status: Completed

There is disease on the farm - if the cause is coming in with the water it is causing disease without any additional stressors. Then, if the hypothesis is correct, the naive animals should succumb. In the planned experiment there are no infected broodstock - the aim of the experiment is to test the in-coming water (not broodstock). Since abalone seem to be particularly susceptible to this virus, the stock from another farm that have not experienced any disease outbreak are presumably uninfected.

The "stress test" would be one way to test for infected (but not showing disease) broodstock. The issue with boodstock is that if they are the natural host then these infected abalone (out in the wild) have survived the infection (=immune?) and can live happily in the non-stressful ocean environment without coming down with disease. Herpesviruses tend to go "latent" but following stress (which compromises the immune response) they can cause disease (e.g. cold-sores and shingles in humans). Transfer to the farm environment may be sufficient stress for the broodstock abs to cause increased virus shedding (and, maybe, disease). This may be one way to test for infected broodstock. To increase the sensitivity of this "stress test" the stressed broodstock can be placed with some naive abalone (30 mm) that have not been exposed previously (presumably) and therefore will be highly susceptible to infection and disease (as evidenced by mass mortality).

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-----Original Message-----
From: Ann Fleming [mailto:annflem@bigpond.net.au]
Sent: Wednesday, 19 April 2006 14:22
To: Crane, Mark (LI, Geelong); Malcolm.Lancaster@dpi.vic.gov.au;
Subject: RE: On farm infection trial

DELETION [s22]

Thanks Mark
So in the trial Mark Gervis is proposing, which will use disease free stock from another farm, is it likely that they will show the disease in 3 mths if the virus is in the incoming water? Or should we add a stress to precipitate the disease in these animals?
Ann

Dr Ann Fleming

Leader
Fisheries Research & Development Corporation
Abalone Aquaculture Subprogram
15 Range Crescent
Alice Springs
NT 0870
ph: 08 8952 1970
fax: 08 8953 3758
email: annflem@bigpond.net.au
website: http://www.frdc.com.au/research/programs/aas/

-----Original Message-----
From: Mark.Crane@csiro.au [mailto:Mark.Crane@csiro.au]
Sent: Wednesday, 19 April 2006 12:56 PM
To: annflem@bigpond.net.au; Malcolm.Lancaster@dpi.vic.gov.au;
Subject: RE: On farm infection trial

DELETION s22 1

My comments concerning stress were directed at broodstock coming in from the wild. These abalone may be infected at low rates (with no disease and seemingly healthy) and by stressing them it may be possible to precipitate disease in these animals or, at least, increase virus shedding with subsequent transmission to other co-habitating naive abalone that would come down with disease.

My concern about the planned experiment is that being quite lengthy (three months duration) there is the risk that quarantine will be broken inadvertently resulting in the wrong conlusion.

In addition, depending on the relative positions of the farm inlet and outlet and the water currents is it possible that effluent water from the farms can be "sucked" back into the farms?

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-----Original Message-----
From: Ann Fleming [mailto:annflem@bigpond.net.au]
Sent: Wednesday, 19 April 2006 11:29
To: Malcolm Lancaster; Crane, Mark (LI, Geelong);
Subject: FW: On farm infection trial

DELETION s22 1

Dear all
I have just one comments on the proposed trial to determine whether the virus is in the incoming seawater. This is based on a comments made to me by Mark Crane last week when discussing quarantine methodology. He commented that it would be preferable to provide a stress to the new stock while in quarantine to try to induce the disease in carriers which may survive the quarantine process but develop the disease once exposed to the stresses on a farm (correct me if I'm not quite remembering correctly Mark). My point is, should Mark stress the test animals also? It may be possible for the test animals to pick up the virus from the oceanic water but not develop the disease within the 3 month period. Or can we detect carriers some other way, perhaps using pathology tests?
regards
Ann

Dr Ann Fleming
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website: http://www.frdc.com.au/research/programs/aas/

s22

DELETION

28

From: Ann Fleming [annflem@bigpond.net.au]
Sent: Wednesday, 19 April 2006 11:29 AM
To: Malcolm Lancaster; Crane, Mark (LI, Geelong); [s22.1]
Subject: FW: On farm infection trial
Attachments: [s22.1] DELETION water study - virus CS (4).doc

Follow Up Flag: Follow up
Flag Status: Completed

Dear all
I have just one comments on the proposed trial to determine whether the virus is in the incoming seawater. This is based on a comments made to me by Mark Crane last week when discussing quarantine methodology. He commented that it would be preferable to provide a stress to the new stock while in quarantine to try to induce the disease in carriers which may survive the quarantine process but develop the disease once exposed to the stresses on a farm (correct me if I'm not quite remembering correctly Mark). My point is, should Mark stress the test animals also? It may be possible for the test animals to pick up the virus from the oceanic water but not develop the disease within the 3 month period. Or can we detect carriers some other way, perhaps using pathology tests?
regards
Ann

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Research Project - Investigating whether the herpes-like virus is originating from the inflow water at Southern Ocean Mariculture (SOM) and Coastal Seafarms (CS).

Introduction:

The point source of a virus affecting abalone stocks at both Southern Ocean Mariculture and Coastal Seafarms is currently unknown. . General observations at both farms show it is possible that the abalone are being infected from the natural incoming water, which is pumped continually from the ocean. The possible transmission modes are through oceanic gastropods harbouring the virus. At SOM it has been observed that the majority of infected tanks are first infected at the inlet.

Objectives:

The aim of this experiment is to prove whether or not the water being pumped into SOM and CS is the origin of this disease. The result of this study will either be positive or negative. No statistical data is required and therefore only one tank is being employed.

Equipment:

Black plastic tank 200l white plastic tank
6 x standard abalone hides
Inflow hose
Tap
Standpipe
Broom
Feed bucket
Virkon S
Unaffected abalone from Great Southern Waters (GSW)

Method:

The experiment will be set up so that it is kept completely quarantined from the rest of the farm. This will take place in isolated rooms which will be disinfected with Virkon S at a rate of 1:100, along with all other equipment. At SOM the experiment will be conducted in the spawning room. At CS it will be conducted in the brood stock conditioning room. Both rooms have not been used for at least two months they have been fully disinfected and dried out. All other equipment will be totally disinfected and dried, or will be new.

100 abalone of approximately 30mm in size will be used by each farm in the experiment, as this age/size class has been most susceptible to the virus. The animals will be fed five times a week and cleaned twice a week every effort will be made to try and replicate commercial practices, so that true production stress is applied in the trial. To maintain full quarantine from the rest of the farm only one person will be assigned to the area. All feeding, cleaning and general maintenance will be carried out first thing in the morning and never after contact with infected areas of the farm. All cleaning and feeding equipment will not leave the room. This trial will be

conducted for three months and any suspicious animals will be preserved in 10% formalin and sent to Department of Primary Industries (DPI) for confirmation of the virus.

Cost: All costs for running the trial will be an in kind expense of SOM and CS. Testing animals for the virus is hoped to be an in kind contribution by DPI.

29

From: Crane, Mark (LI, Geelong)
Sent: Thursday, 20 April 2006 1:46 PM
To: Corbeil, Serge (LI, Geelong); McColl, Ken (LI, Geelong)
Subject: RE: The influence of water dilution factors on the infectivity of the abalone herpes 20-04-06.doc

Follow Up Flag: Follow up
Flag Status: Completed

Thanks, Serge.

I have made some changes - mainly to include a bit more detail for clarification. See what you think.

Mark

Infectivity trial 2 Report 20-...

MARK CRANE Ph.D.
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-----Original Message-----
From: Corbeil, Serge (LI, Geelong)
Sent: Thursday, 20 April 2006 12:51
To: Crane, Mark (LI, Geelong); McColl, Ken (LI, Geelong)
Subject: The influence of water dilution factors on the infectivity of the abalone herpes 20-04-06.doc

<< File: The influence of water dilution factors on the infectivity of the abalone herpes 20-04-06.doc >>
Guys, here is a draft of the abs experiment. Ken as discussed you will have to modify the histo results according to your findings.

Serge

20-04-2006

Abalone herpes-like virus: Influence of virus-contaminated water dilution on disease transmission

Aims

(1) to establish if the virus can transmit through the water column
(2) to determine if dilution of contaminated water can be used as a means of disease control

Methods

Three rooms of the AAHL large animal facility were set up with 100L tanks containing 80L of seawater (ex MAFRI, Queenscliff) each (mean water temperature: 17^{O}C; range: 15.5-18.5^{O}C). During the course of the experiment, in order to maintain water quality, a 50% water change was performed on each tank on every second day. Aeration was provided for each tank. Twelve to thirteen virus-free, healthy, one-year-old (4 to 5 cm shell length) hybrid abalone (ex Great Southern Waters, Indented Head) were placed in each tank.

All control and experimental groups were duplicated.

Room C8 (negative control room) contained (a) a group of untreated abalone and (b) a group of abalone that were sham-injected with DMEM supplemented with 10% foetal bovine serum (medium alone – no virus).

Room C7 (Portland farm water) contained experimental groups with the following farm water concentration:

Group 1---100%

Group 2----10%

Group 3----0.1%

Group 4-0.001%

The water remained in the tanks for 24 hours and then the tanks underwent the regular 50% water changes on alternate days.

Room C6 (positive control co-habitation) contained the following groups:

Group 1: Co-habitation with abalone injected with 0.1 ml of virus inoculum (50L tank; no direct contact between injected and healthy abalone).

Group 2: Co-habitation with sick abalone (ex Portland farm) (no direct contact between sick and healthy abalone).

Group 3: 100% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 4: 10% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 5: 1% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 6: 0.01% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 7: 0.001% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

All dilutions were made with fresh saltwater obtained from MAFRI, Queenscliff. The water remained in the tanks for 24 hours and then the tanks underwent the regular 50% water changes on alternate days.

Results Summary

Morbidity/mortality (17 days post-virus exposure)

(I) Room C8 (Negative control)

The abalone from both negative control groups (no virus exposure) did not present any morbidity or mortality.

(II) Room C7 (Portland farm water).

Group 1 (100% water): 1 moribund abalone in each duplicate tanks at day 8 and 9 post-exposure to farm water. These animals were sampled and processed for histological examination. Results suggest that the abalone did not shown typical pathological signs of a viral disease and are likely to have become moribund due to other factors.

The group 2 (10% water): 1 moribund abalone in one tank at day 12 post-exposure to farm water. The animal was sampled and processed for histological examination. Result suggests that the abalone did not shown typical pathological signs of a viral disease and is likely to have become moribund due to other factors.

Abalone from group 3 and 4 (0.1 and 0.001% water, respectively) did not show any morbidity or mortality during the course of the experiment.

(III) Room C6 (Positive control co-habitation)

The healthy abalone from group 1 (co-habitation with injected abalone) began dying at day 3 post-exposure and were all dead by day 8 post-exposure.

The healthy abalone from group 2 (co-habitation with sick abalone) began dying at day 4 post-exposure and were all dead by day 8 post-exposure.

The abalone from group 3 (100% co-habitation water) began dying at day 3 post-exposure and were all dead by day 10 post-exposure.

The abalone from group 4 (10% co-habitation water) began dying at day 3 post-exposure. At day 17 post-exposure, 86% of the total number of animals were dead.

The abalone from group 5 (1% co-habitation water) began dying at day 8 post-exposure. At day 17 post-exposure, 43% of the total number of animals were dead.

Abalone from group 6 and 7 (0.01 and 0.001% water, respectively) did not show any morbidity or mortality during the course of the experiment.

Moribund abalone from groups 1 to 5 were sampled at various time points during the course of the experiment and were processed for histological examination. Most of the animals showed pathological signs of viral infection.

Conclusions

There is currently no assay available to detect the aetiological agent (herpes virus-like) causing the outbreak of disease in the abalone farms. Therefore, we were unable to confirm the presence of the virus in the farm water used for the infection trial. Consequently, the following possibilities for the absence of morbidity and mortality of abalone are as follows:

1) There was no virus (or few infectious particles) present in the water at the time of sampling (virus titres fluctuate in a farm setting, mainly 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 too low titre to cause a productive infection in the abalone.

Because there is no evidence that the virus was present in the water at the start of the experiment, we can not draw any conclusion on the potential impact of the farm effluent to the local wild molluscs.

With regards to the dilution experiment carried out using co-habitation with sick abalone (room C8), the results indicated that the virus can be transmitted to abalone

through the water column. In addition, the water remained infective to animals even after a 1 in 100 dilution. As the experiment is still on-going, we do not know if the abalone exposed to water containing a higher virus dilution will succumb to disease after day 17 post-exposure.

30

From: Crane, Mark (LI, Geelong)
Sent: Thursday, 20 April 2006 3:41 PM
To: 'Malcolm.Lancaster@dpi.vic.gov.au'
Cc: Corbeil, Serge (LI, Geelong); McColl, Ken (LI, Geelong)
Subject: Abalone trial 2: Up-date Report
Attachments: Infectivity trial 2 Up-date Report 060420.doc

Follow Up Flag: Follow up
Flag Status: Completed

Malcolm,

As promised, here is a summary of the results and our interpretation for the abalone trial which is still on-going. Please call if you wish to discuss.

Cheers

Mark

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20-04-2006

Abalone herpes-like virus: Influence of virus-contaminated water dilution on disease transmission

Aims

(1) To establish if the virus can transmit through the water column
(2) To determine if dilution of contaminated water can be used as a means of disease control

Methods

Three rooms of the AAHL large animal facility were set up with 100L tanks containing 80L of seawater (ex MAFRI, Queenscliff) each (mean water temperature: 17^{O}C; range: 15.5-18.5^{O}C). During the course of the experiment, in order to maintain water quality, a 50% water change was performed on each tank on every second day. Aeration was provided for each tank. Twelve to fourteen virus-free, healthy, one-year-old (4 to 5 cm shell length) hybrid abalone (ex Great Southern Waters, Indented Head) were placed in each tank.

All control and experimental groups were duplicated.

Room C8 (negative control room) contained (a) a group of untreated abalone and (b) a group of abalone that were sham-injected with DMEM supplemented with 10% foetal bovine serum (medium alone – no virus).

Room C7 (Portland farm water) contained experimental groups with the following farm water concentration:

Group 1---100%

Group 2----10%

Group 3----0.1%

Group 4-0.001%

The water remained in the tanks for 24 hours and then the tanks underwent the regular 50% water changes on alternate days.

Room C6 (positive control co-habitation) contained the following groups:

Group 1: Co-habitation of healthy abalone with abalone injected with 0.1 ml of virus inoculum (50L tank; no direct contact between injected and healthy abalone).

Group 2: Co-habitation of healthy abalone with sick abalone (ex Portland farm) (no direct contact between sick and healthy abalone).

Group 3: Healthy abalone held in 100% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 4: Healthy abalone held in 10% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 5: Healthy abalone held in 1% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 6: Healthy abalone held in 0.01% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

Group 7: Healthy abalone held in 0.001% water originating from the co-habitation tanks (water was transferred when healthy abalone from group 2 became moribund).

All dilutions were made with fresh saltwater obtained from MAFRI, Queenscliff. The water remained in the tanks for 24 hours and then the tanks underwent the regular 50% water changes on alternate days.

Results Summary

Morbidity/mortality (17 days post-virus exposure)

(I) Room C8 (negative controls)

There was no morbidity or mortality in abalone from both negative control (no treatment and sham-inoculated) groups.

(II) Room C7 (Portland farm water).

Group 1 (100% water): 1 moribund abalone in each of the duplicate tanks at day 8 and 9 post-exposure to farm water. These animals were sampled and processed for histological examination. Histological examination suggests that neither of the abalone showed lesions typical of the herpesviral disease and they are likely to have become moribund due to other factors.

The group 2 (10% water): 1 moribund abalone in one tank at day 12 post-exposure to farm water. The animal was sampled and processed for histological examination. This has not yet been examined.

Abalone from group 3 and 4 (0.1 and 0.001% water, respectively) did not show any morbidity or mortality during the course of the experiment.

(III) Room C6 (Positive control co-habitation)

The healthy abalone from group 1 (co-habitation with injected abalone) began dying at day 3 post-exposure and were all dead by day 8 post-exposure.

The healthy abalone from group 2 (co-habitation with sick abalone) began dying at day 4 post-exposure and were all dead by day 8 post-exposure.

The abalone from group 3 (100% co-habitation water) began dying at day 3 post-exposure and, by day 10 post-exposure, all abalone (in both duplicate tanks) were dead.

The abalone from group 4 (10% co-habitation water) began dying at day 3 post-exposure. At day 17 post-exposure, 86% of the animals (all abalone in one tank and 72% in the duplicate tank) were dead.

The abalone from group 5 (1% co-habitation water) began dying at day 8 post-exposure. At day 17 post-exposure, 43% of the animals (all abalone in one tank and no mortality in the duplicate tank) were dead.

Abalone from group 6 and 7 (0.01 and 0.001% water, respectively) did not show any morbidity or mortality during the course of the experiment.

Moribund abalone from groups 1 to 5 were sampled at various time points during the course of the experiment and were processed for histological examination. Most of the slides are yet to be examined.

Conclusions

There is currently no assay available to detect the aetiological agent (a suspect herpesvirus) causing the outbreak of disease in the abalone farms. Therefore, we were unable to confirm the presence of the virus in the farm water that was transported from Portland to AAHL for use in the infection trial. Consequently, the following possibilities for the absence of morbidity and mortality of abalone exposed to dilutions of the Portland farm water are as follows:

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.

Because there is no evidence that the virus was present in the water at the start of the experiment, we cannot draw any conclusion on the potential impact of the farm effluent to the local wild molluscs.

With regards to the dilution experiment carried out using water obtained from co-habitation of healthy and sick abalone (LAF room C8), the results indicated that the virus can be transmitted to abalone through the water column. In addition, the water 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). As the experiment is still on-going, we do not yet know if the abalone exposed to water containing a higher virus dilution will develop disease after day 17 post-exposure.

31

From: McColl, Ken (LI, Geelong)
Sent: Thursday, 20 April 2006 5:35 PM
To: 'Malcolm.Lancaster@dpi.vic.gov.au'
Cc: Corbeil, Serge (LI, Geelong); Crane, Mark (LI, Geelong)
Subject: RE: Abalone trial 2: Up-date Report

Follow Up Flag: Follow up
Flag Status: Completed

Malcolm,

Just want to clarify a small typo in the document Mark sent you.

In the last paragraph, the first line should read: "..... (LAF room C6)....."

With regards to the dilution experiment carried out using water obtained from co-habitation of healthy and sick abalone (LAF room C8), the results indicated that the virus can be transmitted to abalone through the water column. In addition, the water 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). As the experiment is still on-going, we do not yet know if the abalone exposed to water containing a higher virus dilution will develop disease after day 17 post-exposure.

You probably picked it up anyway, but just wanted to make sure you weren't confused.

I've just got the first batch of histo back today, and, already, there are some interesting things to see. Once all the histo is ready, I'll dunk the slides out of the secure area, and I'll bring them up to Attwood so that we can look at them together. I imagine it will be at least a week or two before they're all ready.

Ken

From: Crane, Mark (LI, Geelong)
Sent: Friday, 21 April 2006 9:45 AM
To: [s22]; Anthony.Forster@dpi.vic.gov.au; Malcolm.Lancaster@dpi.vic.gov.au; 'Mehdi. Doroudi'
Cc: [s22]
Subject: RE: AAGA statement
Attachments: Industry Statement Virus Apr 06 MC Comment 060421.doc

Follow Up Flag: Follow up
Flag Status: Completed

[s22]

I have some reservations about interpretations of the results from the experiments undertaken at AAHL. Please see attached document with comments inserted at the appropriate place.

Cheers

Mark

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-----Original Message-----
From: [s22]
Sent: Thursday, 20 April 2006 16:38
To: Anthony.Forster@dpi.vic.gov.au; Crane, Mark (LI, Geelong); Malcolm.Lancaster@dpi.vic.gov.au; 'Mehdi. Doroudi'
Cc: [s22]
Subject: AAGA statement

Gentlemen
AAGA has drafted the attached statement for use at the meeting on Monday 24th. The statement we hope to be constructive and lead to a whole industry approach of co-operation. We would like to get your comments on the draft, if any, to ensure that we are factually correct and that we have not missed anything.
Could you please provide comments by midday Friday 21st April.
Yours with thanks,

Mark Gervis
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Southern Ocean Mariculture
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Port Fairy, Vic 3284
Australia
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Mob: 0428 682881

Australian Abalone Growers Association Inc.
PO Box 925
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Email: shane.mclinden@southseasabalone.com.au

13/04/06

Re: Virus in farmed abalone

The farmed abalone industry and the science community continue to devise and implement experiments to expedite the identification of critical factors relating to the virus currently affecting two abalone farms in Victoria. These factors include its origin, its mode of infection and what effect environmental factors have on its ability to affect abalone.

There has already been a significant increase in the understanding of this virus since the first abalone were affected a few months ago.

There is no evidence of the disease on any other farms or in wild abalone. Sampling carried by Fisheries Victoria (FV) of wild abalone populations in close proximity to the infected farms has failed to find any abalone with the disease.

While trials carried out at the Australian Animal Health Laboratories (AAHL) showed that the virus can be passed from sick abalone to healthy abalone, exposing healthy abalone to water from one of the infected farms' effluent ponds did not result in infection. This provided evidence to suggest that wild populations were not at risk through out going water from the farms.

Comment [c1]: This is too strong a comment. There is still a lot we do not know. For instance we do not know the levels of virus in the water and, in particular, in the water that was transferred to AAHL. We do not know the doses required to cause disease. We do not know how long the virus is stable in the water. Until we know what sort of levels of virus are being shed from sick abalone and the stability of the virus in the water it is difficult to make statement like this.While there is no evidence of disease in the wild this does not mean that wild abalone are not infected.

FV advises that there are no public health or food safety implications associated with this incident. Nonetheless, live sales, although safe, have been voluntarily suspended as part of a strict quarantine procedure.

The aggressive nature of the virus in the farming environment has raised significant concern across the sector and has galvanised support to understand and manage the problem. A high priority research project has been initiated to develop ***prevention, containment and recovery*** measures.

To date the two affected farms have taken the following measures to contain the spread of the virus both on farm and beyond.

1. Implemented a voluntary hold on all live abalone movements from affected farms to processors and other farms.
2. Co-operated fully with government agencies in notification of the disease outbreak and provided a number of samples of infected stock from all sections of the farm.
3. Provided samples of effluent water so that infection trials could be conducted under laboratory conditions to determine the threat outgoing water from the farm posed to the local wild abalone population.
4. Engaged Dr Paul Hardy Smith (Aquatic Veterinarian with extensive experience in aquatic animal health and disease) to advise the farms on prevention, containment and recovery strategies.
5. With the assistance of Dr Hardy Smith designed and implemented a thorough biosecurity procedure aimed at containing and preventing the spread of the virus.
6. Destroyed considerable numbers of infected stock as a control measure.

7. Offered fully transparent data and information to the authorities to assist with the compilation of an epidemiology history.
8. Advised all relevant businesses and authorities in the production chain, so that measures could be implemented to avoid further spread of the virus.

Through an industry based levy system and in kind contribution the farming sector has invested substantial time and money into researching abalone disease and abalone health.

Progress to date includes:
1. Development of an ongoing abalone health surveillance program across all major producing farms in the country.
2. Active contribution to development of the national translocation policy for abalone.
3. Active contribution to development of the national abalone disease survey.
4. Instigating a full investigation into the immune response of stressed abalone through a PhD project.

Abalone aquaculture on a global scale now accounts for as much as 40 per cent of supply. Australian abalone farmers are among the leaders in terms of technology, innovation, marketing, nutrition and environmental management. Australian abalone aquaculture production is growing by as much as 30 per cent per year.

Throughout the world, developing aquaculture/agriculture industries have encountered and overcome viral diseases with great success. Often the farming of a species identifies more about the health of that species than has been known before, and what local diseases may affect the species. As such, current research may be of considerable use to the wild caught sector.

AAGA welcomes the support offered by Fisheries Victoria, Department of Primary Industries Victoria, and the Fisheries Research and Development Corporation. It also encourages continued dialog with the wild sector so the entire industry can move forward with developing a better understanding of abalone health and disease and setting in place policies that help strengthen and protect all industry sectors and the environment.

Yours sincerely,

Shane Mclinden

Chairman, Australian Abalone Growers Association

From: McColl, Ken (LI, Geelong)
Sent: Monday, 24 April 2006 8:55 AM
To: Crane, Mark (LI, Geelong); Corbeil, Serge (LI, Geelong)
Subject: Role of mucus in abalone herpesvirus disease

Follow Up Flag: Follow up
Flag Status: Completed

Guys,

I produced the following document over the w/e for your consideration.

Have a read of it, and we can discuss it later this morning (I'm going to the seminar at 9.30, so perhaps we can talk about it after that).

The whole thing relies on maintaining individual abs in small plastic containers (I saw 550 mL containers in the supermarket at the w/e - 5 for ~$2.20). Maybe the abs might not even need water to be aerated under such

Abalone draft proposal.doc

conditions (assuming we change the water every day).

Ken

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The transmission of abalone herpesvirus – the role of mucus
Draft proposal

Introduction

In order to introduce practical control measures for the herpesvirus that has been implicated in the mortality of cultured abalone at a number of sites in Victoria, an elementary understanding of the epidemiology of the disease is required. A number of questions need to be addressed including: (1) is sea-water the source of the virus?, (2) are subclinically-infected abalone capable of excreting virus?, (3) is mucus from infected abalone infectious?, and (4) is the virus labile at different water temperatures, and at different concentrations of sea-water?

Aims

This project focuses on the role of mucus in the transmission of the disease. There are two major aims:

(1) To determine if mucus from moribund, herpesvirus-infected abalone is infectious.

- Mucus from experimentally-infected abalone will be collected, and then susceptible abalone will be (1) inoculated with the mucus, or exposed to it through (2) direct physical contact, or (3) by being held in mucus-contaminated water.

(2) If mucus is found to be infectious, then ways of treating the infectious mucus in order to prevent transmission of disease will be investigated.

- Does treatment of infectious mucus with detergent, disinfectant, or both, prevent subsequent infection of susceptible abalone?
- Does drying of infectious mucus prevent subsequent infection of susceptible abalone?

Methods

Experiment 1: Is mucus from infected abalone infectious?

1. Inject a large number of abalone (*no. to be based on the results of previous experiments, in particular the proportion of moribund abalone in 24 hr period b/n 2-3, or 3-4 dpi*) with a stock preparation of the herpesvirus (supernatant fluid from homogenized ganglia of known infected abalone). This procedure will yield moribund abalone within 2 to 4 days post inoculation, and mucus will be collected from these abalone.

a. Mucus will be pooled, and held at 4 oC in an airtight container for no longer than 24 hrs
b. It may be useful to investigate ways of increasing the amount of mucus produced by moribund abalone, eg, is placing the abalone on a non-porous, but dry, surface sufficient (yields approximately 0.3 mL/abalone), or could a pharmacological agent be used to stimulate production?

2. Set up treatment groups as shown in Table 1.
    a. Abalone will be held individually in 400 mL of aerated sea-water that is changed every two days.
3. Maintain the experiment for 3 weeks during which time all mortalities will be recorded, and moribund abalone will be processed for histopathological examination.
4. Conduct an uncontrolled trial in which 10 susceptible abalone will be placed in 80 L of sea-water containing a mucus-contaminated brush from an affected farm. Change 50% of the water every two days, and maintain the experiment for 3 weeks (if necessary).

Experiment 2: Are there effective treatments of infectious mucus?

1. Collect a pool of infected mucus (as done previously by injecting a large number of abalone with a stock preparation of the herpesvirus).
2. Having collected mucus, experimental groups will be set up as shown in Table 2.
    a. After mixing 0.5 mL of the virus preparation with the appropriate treatment, all treatments (except no. 13) will be held at room-temperature for 5-10 mins (which covers the recommended time for effective action of Virkon).
    b. In group no. 13, 0.5 mL of mucus will be held at room-temp overnight to allow the mucus to dry prior to the addition of 4.5 mL of diluent.
3. Each 5 mL treatment will then be diluted a further $1:10^2$ and $1:10^3$ in PBS in order to dilute the detergent and/or disinfectant (which would otherwise be expected to be toxic if inoculated directly into abalone). Group no. 13 will also be diluted 1:10 (in addition to $1:10^2$ and $1:10^3$).
4. Four abalone will be inoculated for each dilution of each treatment, and abalone will be held individually in 400 mL of aerated sea-water that is changed every 2 days.
5. Maintain the experiment for 3 weeks during which time all mortalities will be recorded, and moribund abalone will be processed for histopathological examination.

The total number of abalone required for each experiment is shown in Table 3. A single 500 mL container will be required for each abalone, ie, 68 in Experiment 1 and 108 in Experiment 2.

Table 1. Treatments to assess the infectivity of mucus from moribund, herpesvirus-infected abalone

Group	Treatment
1	Direct inoculation of naive abalone with stock preparation of virus[1] • Test stock virus: undiluted, 1:10, $1:10^2$, $1:10^3$, $1:10^4$ • Four abalone tested for each dilution, and then held individually in sea-water in 400 mL tanks
2	Direct inoculation of naïve abalone with infected mucus • Test mucus: undiluted, 1:10, $1:10^2$, $1:10^3$, $1:10^4$ • Four abalone tested for each dilution, and then held individually in sea-water in 400 mL tanks
3	Direct physical contact of naïve abalone with infected mucus • Test mucus: undiluted, 1:10, $1:10^2$, $1:10^3$, $1:10^4$ • 0.3 mL of mucus coated on a non-porous surface for each test, and abalone placed on mucus for 5 min. • Abalone then washed with sea-water to remove cell-free virus • Four abalone tested for each dilution, and held individually in sea-water in 400 mL tanks
4	Abalone held in mucus-contaminated sea-water • Prepare 1.6 L of sea-water with mucus at $1:10^3$, and 1.6 L with mucus at $1:10^4$ (also test $1:10^2$ if sufficient mucus available) • Four abalone tested for each dilution, and held individually in mucus-contaminated sea-water in 400 mL tanks for 24 hr

[1]Stock preparation of virus: supernatant fluid from homogenized ganglia of infected abalone.

Table 2. Experimental groups to assess the effect of treatment regimes on virus infectivity

Group	Virus preparation (0.5 mL)	Treatment (4.5 mL)
1	Stock preparation of virus[1]	Tissue-culture medium
2	Stock preparation of virus	Detergent[2]
3	Stock preparation of virus	Disinfectant[3]
4	Stock preparation of virus	Detergent plus disinfectant[4]
5	Virus-free tissue-culture medium	Tissue-culture medium
6	Virus-free tissue-culture medium	Detergent
7	Virus-free tissue-culture medium	Disinfectant
8	Virus-free tissue-culture medium	Detergent plus disinfectant
9	Pooled mucus from moribund abalone	Tissue-culture medium
10	Pooled mucus from moribund abalone	Detergent
11	Pooled mucus from moribund abalone	Disinfectant
12	Pooled mucus from moribund abalone	Detergent plus disinfectant
13	Pooled mucus from moribund abalone – dried[5]	Tissue-culture medium

[1]Stock preparation of virus: supernatant fluid from homogenized ganglia of infected abalone. [2]????. [3]Virkon. [4]Prepared as recommended by manufacturer of Virkon. [5]Mucus dried at room-temperature overnight.

Table 3. The total number of dilutions and abalone required for each experiment

Experiment 1

Group	No. of dilutions per treatment	X no. of abalone per dilution	Total abalone per treatment
1	5	x 4	20
2	5	x 4	20
3	5	x 4	20
4	2	x 4	8
TOTALS			68

Experiment 2

Group	No. of dilutions per treatment	X no. of abalone per dilution	Total abalone per treatment
1	2	4	8
2	2	4	8
3	2	4	8
4	2	4	8
5	2	4	8
6	2	4	8
7	2	4	8
8	2	4	8
9	2	4	8
10	2	4	8
11	2	4	8
12	2	4	8
13	3	4	12
TOTALS			108

From: Crane, Mark (LI, Geelong)
Sent: Friday, 28 April 2006 10:33 AM
To: Elliott, Nick (CMAR, Hobart)
Subject: RE: Abalone health

Follow Up Flag: Follow up
Flag Status: Completed

Nick,

We do not know whether it is the same or similar virus as the Taiwan virus. The histopathology looks similar but we do not have any sequence from either viruses to compare.

Based on current epidemiological analysis the most likely source is from the wild - possibly broodstock abalone from a specific area. The industry is very keen for wild abalone to be tested - but we need a PCR test for that.

The o/s herpesvirus of oysters is from Pacific oysters.

In the experimental infections mortality is 100%.

In the natural infections I know one farm is holding on to survivors (very few - maybe 10-20 from a whole year class) with the thinking that these may have some resistance and may be useful in the future.

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-----Original Message-----
From: Elliott, Nick (CMAR, Hobart)
Sent: Friday, 28 April 2006 09:20
To: Crane, Mark (LI, Geelong)
Subject: RE: Abalone health

Hi Mark

Thank you for the update which filled in some gaps I had from the different industry groups. I have raised the issue with the Flagship to see if they can help out. I have a meeting with them on Monday so will follow it up then.

Some follow-up questions
Is it true that this virus is the same or similar to one previous observed in Taiwan? Is there any evidence to confirm likely source? You mention herpesvirus in oysters overseas, was this in Pacific oysters, in which case do we need to be mindful of possible transfer? Does it affect all animals in a tank or only a proportion?

Cheers
Nick

From: Crane, Mark (LI, Geelong)
Sent: Thursday, 27 April 2006 10:46 AM
To: Malcolm Lancaster (malcolm.lancaster@dpi.vic.gov.au)
Cc: McColl, Ken (LI, Geelong); Hyatt, Alex (LI, Geelong)
Subject: AAHL Diagnsotic report and subsequent publications

Follow Up Flag: Follow up
Flag Status: Completed

Malcolm,

As discussed with you yesterday, I have checked our records and a report (SAN 06-00208) with the em results was forwarded to Bronwyn Murdoch (cc'd to you and Hugh Millar) at the end of January. I have also spoken with Alex and have clarified the situation with him re: Celia Hooper. i.e. DPI Victoria is our client and results should be forwarded to your agency only and not to Gribbles. As we discussed yesterday, Celia may wish to publish her findings but a more comprehensive manuscript with the epidemiology, histo and Alex's em work will be put together between DPI Victoria and AAHL. It is likely that a third publication should be prepared with the transmission trials and sequential development of histo and em coming out of those trials - to be discussed once we have all the results from the experimental infections.

It was a useful day yesterday. This incident may well have put the cat amongst the birds re: funding mechanisms for disease emergencies in aquaculture. It will be interesting to see how it develops.

Cheers

Mark

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From: Crane, Mark (LI, Geelong)
Sent: Thursday, 27 April 2006 12:00 PM
To: Elliott, Nick (CMAR, Hobart)
Cc: Walker, Peter (LI, Geelong)
Subject: RE: Abalone health

Follow Up Flag: Follow up
Flag Status: Completed

Hi Nick,

Thanks for your email with your offer of assistance. To summarise, the outbreaks on farms in SW Victoria started in December 2005. 1- and 2-year-olds are most severely affected. Presumable spat are infected and as they grow on (to a threshold size/age) they seem to develop disease and die. Samples were sent to AAHL in January 2006 for EM examination. The presence of a herpes-like virus (based on ultrastructure) was confirmed. In collaboration with DPI Victoria and industry we have undertaken two infectivity trials to determine the transmissibility of the disease. Basically, we have shown that the disease can be transmitted:

1. By injection of filtered homogenate from fresh, moribund Abs (mortality started 2-3 days pi)
2. By injection of filtered homogenate from frozen abalone samples (mortality started 3-4 days pi)
3. By cohabitation (no contact) with sick abalone (mortality started 4-5 days pi)

Presence of virus has been confirmed by em in the experimental animals. Thus we have a good in vivo model to work with.

In addition, we are looking at a number of PCR primer sets generated for sequencing the oyster herpesvirus from overseas but, to date, we do not have any positive results with the abalone samples - indicating that we would have start from scratch with this new virus.

Together with DPI Victoria and industry we are seeking funds to continue this work. Of course, the incident is completely out-of-cycle wrt FRDC funding. The process for 2006/7 funding has been completed and therefore any new proposal to be submitted to FRDC will be for 2007/8 - a bit late for an on-going outbreak.

Essentially, the affected farms are getting rid of infected stock that are not marketable. Unaffected stock of marketable size are being emergency harvested. Following clearance of stock, the farms will clean up and disinfect in readiness to receive new stock. Of course, they will need to place some sentinel animals in the facilities to determine whether the clean-up has been effective. The farms are also considering some infrastructure development (e.g. pouring concrete floors rather than having sand base) to facilitate clean-up following any future outbreaks. In addition, they will buy in 1- and 2-year-olds from other, unaffected farms for growing on to get them through the next period. What is needed is a diagnostic (PCR-based) technique for surveying broodstock that would be quarantined until testing was complete. Whether this would be used in conjunction with a stress test (with co-habitation with healthy 1-year-olds?) is to be determined. Clearly, we cannot wait for FRDC-funding (with no guarantee of success anyway). We anticipate that this would be at least a 2-year project and needs to be started asap.

Any advice from you would be appreciated.

Cheers

Mark

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-----Original Message-----
From: Elliott, Nick (CMAR, Hobart)
Sent: Wednesday, 26 April 2006 16:07
To: Crane, Mark (LI, Geelong)
Subject: Abalone health

Hi Mark

I gather you are undertaking the research into the 'herpes-like' virus that has become a problem in two of the Victorian abalone farms.

This short note is just to offer you any support I can provide through our projects or the Flagship in general.

Our abalone research is focussed around genetic improvement, so includes breeding program design, and understanding traits such as growth, product quality and health (was minimal until this issue arose). All our abalone work is within the Food Futures Flagship, and so if you need some support let me know, we may be able to entice some fire-fighting funds out of them.

I would also appreciate any feedback you can give me on the virus or what ever it is.

Cheers
Nick

From: Elliott, Nick (CMAR, Hobart)
Sent: Tuesday, 2 May 2006 2:22 PM
To: Lee, Bruce (FF F/ship, North Ryde); Preston, Nigel (CMAR, Cleveland)
Cc: Crane, Mark (LI, Geelong)
Subject: Abalone health research / need for immediate support

Follow Up Flag: Follow up
Flag Status: Completed

Dear Bruce and Nigel

In summer this year the first major health issue for the aquaculture of abalone in Australia occurred. Two farms in Victoria experienced major losses due to the presence of a herpes-like virus.

Some preliminary research has been undertaken by Mark Crane and other CLI colleagues at AAHL – ultrastructure confirmation of herpes-like virus, confirmation of high infectivity, and testing of published herpesvius species PCR primers for identification (unfortunately no amplification).

The national industry is rightly very concerned about the ramifications of this outbreak, particularly as the suspected source of infection (not proved) was with transferred wild broodstock. Other possibilities include with feed. There are other implications for transfer of other farmed stock within and between States, and in regards to selective breeding.

The industry has placed research on this issue as it highest immediate priority and Mark Crane is working with DPI Victoria and industry to attract FRDC funds. Unfortunately, the outbreak occurred out of the usual FRDC funding cycle so it is uncertain whether funds can become available in 06/07. Immediate research needs include developing a diagnostic (PCR-based) protocol for screening potential broodstock for the 06/07 season, as well as setting up suitable quarantined research facilities for testing and challenging to further understand the problem.

Given the urgency in this matter, would the FFF consider providing some immediate support to Mark to enable essential R&D to commence before further industry/FRDC co-investment is possible?

Thank you and regards

Nick

From: Crane, Mark (LI, Geelong)
Sent: Monday, 8 May 2006 11:46 AM
To: 'annflem@bigpond.net.au'
Cc: Corbeil, Serge (LI, Geelong)
Subject: RE: Abalone Pathogen Pre-proposal from SARDI

Follow Up Flag: Follow up
Flag Status: Completed

Ann,

We do intend to submit this year and we will send you the preproposal when it is finalised.

Cheers

Mark

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-----Original Message-----
From: Ann Fleming [mailto:annflem@bigpond.net.au]
Sent: Monday, 8 May 2006 11:43
To: Crane, Mark (LI, Geelong)
Cc: [s22]
Subject: RE: Abalone Pathogen Pre-proposal from SARDI]

DELETION

Mark
Thanks for responding to the SARDI submission. Regarding your plan to submit funds to continue your work on PRC primers for the abalone virus. I am currently working with industry at present assisting with developing their R&D strategy. A detection tool for the virus is a high priority of course. I planned to talk to you regarding how to get it funded and that perhaps it should go through the AAH SP. Do you intend to submit for this year and, if so, could you keep me informed on your progress and send the preproposal when its available.

Thanks Mark
regards
Ann

Dr Ann Fleming
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Abalone Aquaculture Subprogram
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website: http://www.frdc.com.au/research/programs/aas/

From: Corbeil, Serge (LI, Geelong)
Sent: Monday, 8 May 2006 3:14 PM
To: Crane, Mark (LI, Geelong)
Subject: FRDC Ab herpes virus 08-05-2006 preproposal.doc
Attachments: FRDC Ab herpes virus 08-05-2006 preproposal.doc

Follow Up Flag: Follow up
Flag Status: Completed

AQUATIC ANIMAL HEALTH SUBPROGRAM

2007-08 Preliminary Research Proposal

Note: Phrases in italics are for clarification only and should be deleted after the form is filled in.
Format - 10pt font minimum, 3 pages maximum!

Project Title *All titles to commence with 'Aquatic Animal Health Subprogram:'*

Aquatic Animal Health Subprogram: Molecular characterisation of an emerging abalone herpes-like virus

FRDC Strategic challenge identification

Natural Resources Sustainability

Principal Investigator Contact Details

Title: Dr Serge Corbeil
Organisation: CSIRO
Mailing Address: Private Bag 24, Geelong, Vic, 3220
Phone No: 03 52275254 Fax No: 03 52275555 Email: serge.corbeil@csiro.au

Commencement and completion date

Commencement date:01-02-2007
Completion date: 31-01-2009

Preliminary Budget *(insert $$s)*

FRDC Contribution	2007-08	2008-09	2009-10	2010-11	TOTAL $$s
Salaries and on costs					
Travel					
Operating					
Capital (equipment)					
FRDC TOTAL					
Research Organisation contribution					
Total of Industry & Other Funding					
GRAND TOTAL in $$s					

Need

In December 2005/January 2006, a disease outbreak caused high mortality rates in abalone from two farms in Victoria. A third Victorian farm also experienced disease but to a lesser extent. The abalone species affected by the outbreak are *Haliotis laevigata*, *H. rubra, and H. laevigata* x *H. rubra* hybrid. Histopathology performed on moribund animals indicated a ganglioneuritis – infiltration of haemocytes in multiple ganglia and nerves (cerebral ganglion affected, also other ganglia and nerves). Examination by electron microscopy revealed the presence of a herpes-like virus in the pleuropedal ganglion. Preliminary transmission studies, carried out within AAHL's high biosecurity facility, indicated that this emerging virus is highly pathogenic and can be transmitted to healthy abalone through the water column. In addition, the viral suspension remains infectious after undergoing a dilution up to 1 in 100. So far, the virus has not grown in tissue culture.
Attempts by the farm managers to eliminate the disease from the abalone farms have failed partly due to the lack of detection methods specific to the aetiological agent which would allow early diagnosis of infected animals. If not managed appropriately, this emerging virus has the potential to spread within Victorian abalone farms as well as to wild abalone broodstock and to farms in other States and would jeopardise Australia's high market-value abalone industry.

Access to diagnostic tests that are rapid, reliable and sensitive is of fundamental importance for effective control/management of disease outbreaks. Hence, laboratories around the world are now developing disease-monitoring programs based on molecular (DNA) diagnostic techniques for the most economically important mollusc pathogens. The

molecular characterisation of this emerging abalone virus and the development of a quantitative molecular assay will provide researchers and industry with a tool that can rapidly and specifically detect the virus in abalone tissues therefore providing a vital means for diagnosis and control.

Objectives

1. To characterise the emerging abalone virus at the molecular level (gene amplification and sequencing).
2. To develop and validate a real-time TaqMan PCR assay for the detection of the abalone virus.
3. To determine which abalone tissues provide accurate diagnosis of infection.
4. To document an Australian and New Zealand Standard Diagnostic Procedure and submit for external review.

Industry and Management consultation

The abalone Growers Association of Victoria strongly supports the proposal.
VicFRAB
Abalone Subprogram
AAH Subprogram
SAFRAB
WAFRAB
Tas FRAB

Direct benefits and beneficiaries

1. The diagnostic test will be available to detect and identify the emerging virus in abalone (*Haliotis* spp.), and other potential host mollusc species.

2. Export certification services will be available to industries that wish to develop export markets as well as translocate farmed stock between regions without transmitting disease. In addition, should pathogenic agents be detected during health surveys, industries and State officers will be able to make informed decisions with regards to brood stock translocation, stock destruction etc. Specifically, mollusc aquaculture industries and State agencies will be able to develop health surveillance programs in collaboration with AFDL. In addition, Australia will be better prepared to negotiate with international trading partners on issues concerned with the importation of disease free molluscs from Australian sources.

Estimated Flow of Benefits

Fisheries and aquaculture managed by States/Territories	
NSW	5%
Qld	1%
SA	15%
Tas	49%
Vic	25%
WA	5%
AFMA managed fisheries	0%
Other beneficiaries	0%
Total for all fisheries	100%

Project Design and Methodology

-The subtractive in situ hybridisation method will be use to clone viral gene fragments from infected abalone tissues.
-Cloned genes (fragments) will be sequenced and blasted against gene data banks in order to find existing homologous viral genes.
-Primers will be synthesized to perform a walking PCR strategy in order to get appropriate gene length for the development of a real-time PCR assay.
-Using the Primer Express Sofware version 1.5 (PE Applied Biosystems), primers and probes will be designed to detect the abalone virus.
-Infection trials of healthy abalone will be performed under biosecurity facility, sampling and testing of various abalone tissues will be conducted.

-Procedures for the detection and identification of the abalone virus will be incorporated into an Australian and New Zealand Standard Diagnostic Procedure (ANZSDP) and submitted to SCAHLS for review and publication.

Research Capability and Experience

Serge Corbeil: BSc MSc PhD. Eleven years experience in aquatic animal disease research and diagnosis (viral, protozoan and bacterial diseases). Nine years experience in molecular diagnosis (conventional PCR, real-time PCR, gene sequencing) and immunodiagnosis of aquatic animal diseases. Five years experience in vaccine R&D for mammalian and fish diseases.

Previous FRDC Projects

Corbeil, S. and Crane, M. St. J. Aquatic Animal Health Subprogram: development of diagnostic procedures for the detection and identification of *Piscirickettsia salmonis*. Fisheries Research and Development Corporation Project Number 2001/624.

Corbeil, S. and Crane, M. St. J. Aquatic Animal Health Subprogram: Development of molecular diagnostic expertise for the mollusc pathogen *Bonamia* sp. Fisheries Research and Development Corporation Project Number 2003/622.

Relevant Publications

Corbeil, S., McColl, K. A. and Crane, M. St. J. (2003) Development of a TaqMan quantitative PCR assay for the identification of *Piscirickettsia salmonis*. *Bull. Eur. Ass. Fish Pathol.* 23: 95-101.

Corbeil, S., Hyatt, A. D. and Crane, M. St. J. (2005) Characterisation of an emerging rickettsia-like organism in Tasmanian farmed Atlantic salmon *Salmo salar*. *Dis. Aquat. Org.* 64: 37-44.

Corbeil, S., Arzul, I., Robert, M., Berthe, F. C. J., Besnard-Cochennec, N. and Crane, M. St. J. (2006) Molecular characterisation of an Australian isolate of *Bonamia* isolate from *Ostrea angasi*. *Dis. Aquat. Org.* In press.

Corbeil, S., Arzul, I., Diggles, B., Heasman, M., Chollet, B., Berthe, F. C. J. and Crane, M. St. J. (2006) Development of a TaqMan PCR assay for the detection of *Bonamia* species. *Dis. Aquat. Org.* In press.

Please forward the Preliminary Research Proposal in a MS-Word format via e-mail to:

Ms Joanne Slater
Coordinator
Aquatic Animal Health Subprogram
c/o AAHL Fish Diseases Laboratory
Australian Animal Health Laboratory
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No later than cob on Friday 23 June 2006.

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From: Crane, Mark (LI, Geelong)
Sent: Monday, 8 May 2006 12:57 PM
To: Malcolm Lancaster (malcolm.lancaster@dpi.vic.gov.au)
Cc: Mehdi Doroudi (mehdi.doroudi@dpi.vic.gov.au); Corbeil, Serge (LI, Geelong); Walker, Peter (LI, Geelong)
Subject: FW: Gribbles and PRC primer work

Follow Up Flag: Follow up
Flag Status: Completed

FYI

It looks as if everybody is trying to jump on the bandwagon. I have spoken with Mark Gervis and suggested to him that since DPI Vic are responsible for disease management we need to ensure that the work is coordinated through DPI Vic otherwise there will be wasteful duplication. I find it difficult to believe that Gribbles could get a PCR up and running from scratch within 2-3 weeks.

Mark

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-----Original Message-----
From: Mark Gervis [mailto:mg@som-abalone.com.au]
Sent: Monday, 8 May 2006 12:47
To: Crane, Mark (LI, Geelong)
Subject: FW: Gribbles and PRC primer work

-----Original Message-----
From: Ann Fleming [mailto:annflem@bigpond.net.au]
Sent: Friday, 28 April 2006 4:27 PM
To: Mark Gervis
Subject: RE: Gribbles and PRC primer work

Mark
Gribbles is doing some work on PCR primers for the ab virus. Their vet virologist in Adelaide David Tisdall (0882023308, david.tisdall@gribbles.com.au) does the routine testing in SA but when he gets time he spends it on R&D. Their attitude is that they tend to look at developing new primers when a new issue appears and then they can offer a diagnostic service and testing program. But he fits the work around the commercial side of things and so is a bit unfocussed.

He has done some preliminary work on samples given to him by Celia Hooper when she visited Tim's farm last year. He has tested some primers based on those used by the French people on oysters but hasn't had any success yet. He plans to use different genes and sets of primers next. He thinks he can do this in the next 2-3 weeks. So the situation is that the work is pretty adhoc but he is keen to gear it up when time permits. He is in periodic contact with Mark Crane and knew he was working on PCR primers as well.

I asked him if it is likely that he will be able to develop a product in the near future that will be commercially available to the industry. He says he will be able to say more clearly in 2-3 weeks after he has tested the next range of off-the-shelf PCR primers. So I'll talk to him again then.

He is struggling with only a couple of ab samples and would appreciate more. Ten samples of tissue taken from around the mouth region either frozen or on ice. His address is on its way. regards Ann

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