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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 2 – Documents 41-50

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

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From: Crane, Mark (LI, Geelong)
Sent: Monday, 8 May 2006 1:03 PM
To: [s22] DELETION
Subject: FW: Farm visit to discuss future research
Attachments: Abalone draft proposal-mucus 060424.doc; Abalone draft proposal-mucus 060424.pdf

Follow Up Flag: Follow up
Flag Status: Completed

[s22] DELETION

Resending previous draft as well as a pdf copy of it. Please let me know when you receive it and if you can open up the attached files.

Cheers

Mark

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

International Phone: +61 3 52 275118
International Fax: +61 3 52 275555
email: mark.crane@csiro.au

-----Original Message----- DELETION
From: Crane, Mark (LI, Geelong)
Sent: Thursday, 27 April 2006 10:25
To: [s22]
Cc: McColl, Ken (LI, Geelong); Malcolm Lancaster (malcolm.lancaster@dpi.vic.gov.au)
Subject: RE: Farm visit to discuss future research

[s22]

The draft proposal is attached. I have not started revising it following our discussions yesterday but at least this is a good starting point for your needs - good luck with the insurance people.

Cheers

Mark

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

International Phone: +61 3 52 275118
International Fax: +61 3 52 275555
email: mark.crane@csiro.au

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

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.

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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).
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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
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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.

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9	2	4	8
10	2	4	8
11	2	4	8
12	2	4	8
13	3	4	12
TOTALS			108

42

From: Crane, Mark (LI, Geelong)
Sent: Wednesday, 10 May 2006 12:25 PM
To: Corbeil, Serge (LI, Geelong)
Subject: RE: FRDC Ab herpes virus 08-05-2006 preproposal.doc
Attachments: FRDC Ab herpes virus 08-05-2006 preproposal MCRev 060510.doc; research costing abs herpes 060510.xls

Follow Up Flag: Follow up
Flag Status: Completed

Serge,

I have reviewed and edited the preproposal. I have also prepared the budget sheets for the project as described. I still think that this may need some work. For example, it is unlikely that you will be working on this project only and so it will not take 100% of your time. Also others such as Nette and Ken (me?) will be participating (our time may be in-kind and therefore boost the AAHL component of the budget which FRDC will like) - at least for any infection trials. Reducing your time and adding Nette (Nette has indicated that she wishes to gain some molecular skills) may reduce the overall budget. If Peter gets back before me discuss this with him so that we can get it out to the various FRABs asap (the dates for preproposals to the FRABs can be found on the FRDC website). The Aquatic Animal Health Subprogram will not need it until June.

I have left the budget as a non-secure Geelong project because it maybe that we can do infections in the final year non-secure too.

See you when I get back

Cheers

Mark

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

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email: mark.crane@csiro.au

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	2006-07	2007-08	2008-09	2009-10	TOTAL $$s
Salaries and on costs	48 936	101 787	112 287	61 733	324 743
Travel		1 000	1 000		2 000
Operating	16 064	27 213	26713	13 267	83 257
Capital (equipment)					
FRDC TOTAL	**65 000**	**130 000**	**140 000**	**75 000**	**410 000**
Research Organisation contribution	36 079	75 645	85 188	44 897	241 810
Total of Industry & Other Funding	x	x	x	x	x
GRAND TOTAL in $$s					**651 810**

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. The use of these molecular tools will also facilitate a better understanding of the epidemiology of this disease, leading to more efficient management of disease outbreaks.

Objectives

1. To characterise the emerging abalone virus at the molecular level (via PCR-based 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 underwithin the AAHL biosecuritye facility, to determine which sampling and testing of various abalone tissues are the most appropriate for sampling during an active surveillance program of, for example, wild broodstock, 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
CSIRO Livestock Industries
Private Bag 24, Geelong VIC 3220

Phone: 03 5227 5427
Fax: 02 5227 5555
E-MAIL: joanne.slater@csiro.au
Website: http://www.frdc.com.au/research/programs/aah/index.htm

No later than cob on Friday 23 June 2006.

version 7/2/06							
FUNDING ORGANISATION							
START DATE							
PROJECT							
Site		Geelong					
		2005/06	2006/07	2007/08	2008/09	2009/10	TOTAL
SALARIES							
NAME	LEVEL	SALARY	SALARY	SALARY	SALARY	SALARY	SALARY
		0	39149	81429	89829	49386	259794
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
DIRECT COSTS							
salary		0	39149	81429	89829	49386	259794
onc		0	9787	20357	22457	12347	64948
total salary		0	48936	101787	112287	61733	324742
TO… STAFF		0.00	0.50	1.00	1.00	0.50	
travel				1000	1000		2000
materials			10000	15000	15000	5000	45000
other ie PCR, DNAseq etc							0
animals							0
TOTAL DIRECT COSTS		0	58936	117787	128287	66733	371742
DIRECT OVERHEADS							
Photography ($50 per hour)				200	200		400
Animal Services		0	0	0	0	0	0
Engineering & Site Services							0
Scientific services							
Depreciation equipment		0	3572	7430	8197	4506	23706
TOTAL DIRECT OVERHEADS		0	3572	7630	8397	4506	24106
INDIRECT OVERHEADS							
Executive management		0	4894	10179	11229	6173	32474
Finance		0	1796	3736	4121	2266	11918
People Development		0	1365	2840	3133	1722	9060
OH&S		0	891	1853	2044	1124	5910
Computing		0	1551	3227	3559	1957	10294
Commercial operations		0	2178	4530	4997	2747	14451
Marketing/Communications		0	940	1954	2156	1185	6235
Records Management		0	621	1293	1426	784	4124
Libr…		0	1571	3267	3604	1982	10424
Se…		0	568	1181	1303	716	3767
Mic… curity		0	0	0	0	0	0
Quality Assurance		0	0	0	0	0	0
Lab services		0	377	784	865	475	2501
Animal Services- availability		0	3719	7736	8534	4692	24680
Store		0	1752	3644	4020	2210	11626
Maintenance/Site Services		0	4962	10321	11386	6260	32929
Utilities		0	915	1903	2100	1154	6073
Animal Ethics		0	411	855	943	519	2728
Other(incl photo/reception/overheads)		0	274	570	629	346	1819
Depreciation AAHL Building		0	4404	9161	10106	5556	29227
TOTAL INDIRECT OVERHEADS		0	33188	69032	76153	41867	220240
CORPORATE OVERHEADS		0	5383	11197	12352	6791	35722
TOTAL COSTS		0	101079	205645	225188	119897	651810
COMPETITIVE NEUTRALITY ONCOST FACTOR		0	15364	31258	34229	18224	99075
EXTERNAL FUNDING			65000	130000	140000	75000	410000
EXTERNAL FUNDING % TOTAL COSTS		#DIV/0!	64%	63%	62%	63%	63%
IN-KIND CONTRIBUTIONS		0	36079	75645	85188	44897	241810
MULTIPLIER		#DIV/0!	2.58	2.53	2.51	2.43	2.51
SIGNED		SIGNED				SIGNED	
PROJECT LEADER		COMMERCIAL MANAGER				DELEGATE	

version 7/2/06

	NAME	2005/06 CSOF	%	2006/07 CSOF	%	2007/08 CSOF	%	2008/09 CSOF	%	2009/10 CSOF	%
employee 1	Serge Corbeil	1.1	0%	5M	50%	5M	100%	6.1	100%	6.2	50%
employee 2		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 3		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 4		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 5		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 6		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 7		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 8		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 9		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 10		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 11		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 12		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 13		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 14		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
employee 15		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%

Rocky, Armidale, Floreat Pa

	YEAR 1 rate per day	no. of days	no. of anima	total	YEAR 2 rate per day	no. of days	no. of anin	total	YEAR 3 rate per day	no. of days	no. of ani	total	YEAR 4 rate per da	no. of	no. of anin	total	YEAR 5 rate per da	no. of days	no. of anin	total
cattle	4.3			0	4.3			0	4.3			0	4.3			0	4.3			0
sheep,goats,pigs	0.86			0	0.86			0	0.86			0	0.86			0	0.86			0
poultry	0.43			0	0.43			0	0.43			0	0.43			0	0.43			0
other	0			0	0			0	0			0	0			0	0			0
				0				0				0				0				0

Belmont, Yalanbee, Chiswic

	YEAR 1 rate per hour	no. of hours	total	YEAR 2 rate per hour	no. of hours	total	YEAR 3 rate per hour	no. of hours	total	YEAR 4 rate per hc	no. of hours	total	YEAR 5 rate per hc	no. of hours	total
	45		0	47		0	49		0	51		0	53		0

Large Animal Facility, Geelo

	YEAR 1 Rate per day	No.of days	No.of rooms	total	YEAR 2 Rate per day	No.of days	No.of roon	total	YEAR 3 Rate per day	No.of days	No.of roc	total	YEAR 4 Rate per d	No.of	No.of roon	total	YEAR 5 Rate per d	No.of days	No.of roon	total
	157			0	164			0	172			0	181			0	190			0

Small Animal Facility, Geelo

	YEAR 1 rate per box per day	no. of days	No. of boxes	total	YEAR 2 rate per box pe	no. of days	No. of box	total	YEAR 3 rate per box p	no. of days	No. of bo	total	YEAR 4 rate per bc	no. of	No. of box	total	YEAR 5 rate per bc	no. of days	No. of box	total
Rabbits	16.00			0	16.80			0	17.64			0	18.52			0	19.45			0
Poultry	20.00			0	21.00			0	22.05			0	23.15			0	24.31			0
Poultry SPF	20.00			0	21.00			0	22.05			0	23.15			0	24.31			0
Rats	4.00			0	4.20			0	4.41			0	4.63			0	4.86			0
Mice	3.00			0	3.15			0	3.31			0	3.47			0	3.65			0
				0				0				0				0				0

Werribee

Paddock	$22 per day
Building 5 - small rooms	$94 per day
Building 5, 7 or 8	$188 per day

Animal accomodation	YEAR 1 No. of rooms	rate per day	No. of days	total	YEAR 2 No. of rooms	rate per da	No. of day	total	YEAR 3 No. of rooms	rate per da	No. of da	total	YEAR 4 lo. of room	rate pe	No. of day	total	YEAR 5 lo. of room	rate per da	No. of day	total
Paddock		22		0		22		0		22		0		22		0		22		0
Building 5 - small		94		0		94		0		94		0		94		0		94		0
Building 5, 7 or 8		188		0		188		0		188		0		188		0		188		0
				0				0				0				0				0

DAYS CONVERTER

DAYS	%
2	0.0091

HOURS	
0	0.0000

A.1	19202
A.2	24001
A.3	28802
AM	34562
1.1	28867
1.2	29399
1.3	30130
1.4	30862
1.5	31594
1M	32325
2.1	33554
2.2	34766
2.3	35977
2.4	37189
2.5	38402
2.6	39614
2.7	40827
2.8	42039
2M	43250
3.1	44808
3.2	46354
3.3	47878
3.4	49403
3.5	50929
3.6	52454
3.7	53980
3.8	55504
3M	57028
4.1	58065
4.2	60901
4.3	62838
4.4	64774
4M	66711
5.1	69569
5.2	72428
5M	75286
6.1	79858
6.2	84431
6.3	89004
6M	93578
7.1	98134
7.2	98691
7.3	101247
7.4	103803
7M	106363
8.1	114709
8.2	123055
8.3	133538
8M	144020
9.1	150367
9.2	156713
9.3	163060
9M	169404

Salary scales current as at 13/11/05

version 7/2/06

PRICING DECISION STATEMENT

CONTRACT:
CUSTOMER:
PROJECT: *0*

Type of Activity
- ○ Consulting or Technical Service
- ○ Research - Contract
- ◉ Research - Collaborative - subject to CN
- ○ Research - Collaborative - NOT subject to CN

COSTS:	Direct Costs (less Costs of Sub-contracts)	**395,848**		
	Indirect Costs	**255,962**		
	Cost of Sub-Contracted Activities	-		
	Full-cost Price	**651,810**		
	Competitive Neutrality	99,075		
	Full-cost Price plus competitive neutrality	750,885		
PRICE:	**Cash Contribution**	**410,000**	104% DIRECT COSTS ;	63% TOTAL COSTS
	CSIRO - Livestock Industries In-kind Contributions	**241,810**		
	Value of Other Considerations (1)			

Justification (if applicable) (2):

Prepared by: _____ **Recommended by:** www
Project Leader — Commercial Manager

Approved by: _____ **Date:** _____
Delegate

NOTES:

(1) Other forms of Consideration include long term value of licence fees and royalties
(2) Justifications for prices lower than Full-cost Price include National Interest (specify it) and commercial considerations

version 7/2/06			2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
			0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
FUNDING ORGANISATION		0													
START DATE		0													
PROJECT		0													
Site		Geelong													
			2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
			0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
SALARIES															
NAME			SALARY		SALARY		SALARY		SALARY		SALARY			SALARY	
	☑		0	0	0	39149	0	81429	0	89829	0	49386		0	259794
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
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0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
0	☐		0	0	0	0	0	0	0	0	0	0		0	0
0	☑		0	0	0	0	0	0	0	0	0	0		0	0
DIRECT COSTS															
salary			0	0	0	39149	0	81429	0	89829	0	49386		0	259794
oncosts			0	0	0	9787	0	20357	0	22457	0	12347		0	64948
total salary			0	0	0	48936	0	101787	0	112287	0	61733		0	324742
TOTAL STAFF			0.00		0.50		1.00		1.00		0.50			3.00	
travel	☑		0	0	0	0	0	1000	0	1000	0	0		0	2000
materials	☑		0	0	0	10000	0	15000	0	15000	0	5000		0	45000
other ie PCR, DNAseq etc	☐		0	0	0	0	0	0	0	0	0	0		0	0
animals	☐		0	0	0	0	0	0	0	0	0	0		0	0
TOTAL DIRECT COSTS			0	0	0	58936	0	117787	0	128287	0	66733		0	371742
DIRECT OVERHEADS															
Photography ($40 per hour)	☑		0	0	0	0	0	200	0	200	0	0		0	400
Animal Services	☑		0	0	0	0	0	0	0	0	0	0		0	0
Engineering & Site Services	☑		0	0	0	0	0	0	0	0	0	0		0	0
Scientific services	☐		0	0	0	0	0	0	0	0	0	0		0	0
Depreciation equipment	☐		0	0	3572	0	7430	0	8197	0	4506	0		23706	0
TOTAL DIRECT OVERHEADS			0	0	3572	0	7430	200	8197	200	4506	0		23706	400
INDIRECT OVERHEADS ☐															
Executive management	☐		0	0	4894	0	10179	0	11229	0	6173	0		32474	0
Finance	☐		0	0	1796	0	3736	0	4121	0	2266	0		11918	0
Human Resources	☐		0	0	1365	0	2840	0	3133	0	1722	0		9060	0
OH&S	☐		0	0	891	0	1853	0	2044	0	1124	0		5910	0
Computing	☐		0	0	1551	0	3227	0	3559	0	1957	0		10294	0
Commercial operations	☐		0	0	2178	0	4530	0	4997	0	2747	0		14451	0
Communications	☐		0	0	940	0	1954	0	2156	0	1185	0		6235	0
Records Management	☐		0	0	621	0	1293	0	1426	0	784	0		4124	0
Library	☐		0	0	1571	0	3267	0	3604	0	1982	0		10424	0
Security	☐		0	0	568	0	1181	0	1303	0	716	0		3767	0
Micro security	☐		0	0	0	0	0	0	0	0	0	0		0	0
Quality Assurance	☐		0	0	0	0	0	0	0	0	0	0		0	0
Lab services	☐		0	0	377	0	784	0	865	0	475	0		2501	0
Animal Services- availability	☐		0	0	3719	0	7736	0	8534	0	4692	0		24680	0
Store	☐		0	0	1752	0	3644	0	4020	0	2210	0		11626	0
Maintenance/Site Services	☐		0	0	4962	0	10321	0	11386	0	6260	0		32929	0
Utilities	☐		0	0	915	0	1903	0	2100	0	1154	0		6073	0
Animal Ethics	☐		0	0	411	0	855	0	943	0	519	0		2728	0
Other(incl photo/reception/overheads)	☐		0	0	274	0	570	0	629	0	346	0		1819	0
Depreciation AAHL Building	☐		0	0	4404	0	9161	0	10106	0	5556	0		29227	0
TOTAL INDIRECT OVERHEADS			0	0	33188	0	69032	0	76153	0	41867	0	0	220240	0
			0		0		0		0		0			0	0
CORPORATE OVERHEADS	☐		0	0	5383	0	11197	0	12352	0	6791	0		35722	0
			0		0		0		0		0			0	0
TOTAL COSTS			0	0	42144	58936	87659	117987	96701	128487	53164	66733		279668	372142
EXTERNAL FUNDING			0		65000		130000		140000		75000			410000	

43

From: Matthew Barwick [Matthew@frdc.com.au]
Sent: Tuesday, 16 May 2006 9:31 AM
To: malcolm.lancaster@dpi.vic.gov.au
Cc: Crane, Mark (LI, Geelong); [redacted]
Subject: Re Virus work
Attachments: Judith Handlingers comments for Malcolm on virus work.doc

DELETION s22 1

Follow Up Flag: Follow up
Flag Status: Completed

Malcolm
I understand Mark Gervis will ring you today to confirm his support for the original objectives for the out of cycle application submitted as a draft some weeks ago. So I am sending Judith Handlinger's comments on this draft to you so we can progress it. She makes some points regarding clarifying the objectives of the work and some additional suggestions on methodology.

I would like to offer my assistance in filling in some of the other sections of the application, such as ntroduction, need, industry consultation, etc. I will talk to Crispian Ashby today about what other sections we need to address. Is this OK with you?

Regards
Ann

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?, *Not really the question being asked.*

(2)(2) are subclinically-infected abalone capable of excreting virus?, (*A good question, but not one I think is answered in* ***this*** *proposal*)

(3)(3) is mucus from infected abalone infectious?, and *(Have enough information already to assume it is – not really the question)*

(4)(4) is the virus labile at different water temperatures, and at different concentrations of sea-water? *(Another good question, but not addressed by this proposal).* ***Could possibly add freshwater to your list of treatments in experiment 2 –*** *(then dilute again by sea-water before re-exposure)*

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Herpes virus neuritis is a newly recognised acute viral disease with high mortality which threatens both farmed and wild abalone industries in Australia. Initial attempts to control the virus by partial de-stocking and biosecurity measures on farms were followed by a reduction in new cases, followed by breakdown of control measure on farms and disease recurrence. Initial transmission experiments demonstrated that the virus was highly virulent with a short pre-clinical phase following experimental challenge, was transmitted via water, and survived freezing.

Critical factors for further efforts to control the virus is the source and likelihood of on-going or recurring infection. This involved knowledge of current source of virus associated with disease recurrence, and the resistance of the virus to environmental factors and disinfectants. This will be critical to assessing whether control efforts through further stock reduction or de-stocking will succeed, or be unsuccessful due to virus persistence or a reservoir of infection beyond the scope of the control effort.

It is already known that the virus can be transmitted from live animals through the water column. This study aims to determine the resistance of the virus. Related work includes a study of the effect of dilution on infectivity (that is, assessment of the minimum infective dose) that is already under-way but not yet complete (can we get this from AAHL and include?), and additional studies on-farm studies for indications of whether recurrence and persistence is due to re-infection from incoming water (implying possible external reservoir or infection), or to spread from within the farm (SOM).

Studies of virus resistance will be directed to excreted mucus. The main body excretions of live animals consist of faeces, urine and mucus. As the known virus related lesions are in nervous tissue, there is no reason to suspect faeces would be a specific source of excretion. Urine and any direct haemolymph loss (and this would mostly be mucus associated), would readily be subject to dilution factors. Mucus is likely, therefore, to be the most likely source of concentrated virus in water outflows and tank surfaces, and by its nature may further protect the virus from degradation by

environmental factors such as drying, and by disinfectants. Mucus is therefore the most suitable excretory product to evaluate virus degradation and disinfection.

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. *(well we already know that it really as is likely to be the main excretion from abs which have already been shown to shed into the environment)*

- 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. *Not sure I would bother with direct innoculation of mucus unless the direct contact fails as this is not a normal route of exposure and the effect of inoculation of mucus is an unnecessary study in itself. Would need to compare with animals inoculated with mucus from normal animals. What does this add? Just do 2 and 3, or even one of these - which ever you would see as most practical for the disinfection experiments.*

(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 *Should start with this but may be very useful to freeze – know the virus will survive this. This is probably the only way to ensure that can compare results from successive experiments – and trying to do all of them simultaneously should be thought through very well before attempting. The aim is not to know about the mucus, but about the survival of the virus.*

    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? *Actually probably shaking in plastic bags is the best way – I think this has been done before. Anaesthetic and similar*

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irritants also stimulate mucus production, but need to know if contributing to disinfection. I.e it may be the alcohol the anaesthetic is dissolved in that affects virus survival, and if this is shown to be ineffective, then may be suitable.

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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. *In 2 days will get a lot more new mucus – need to be aware this may confound the statistical assessment of how many became infected by the primary inoculum, but not the main objective of determining if infection can be spread this way. A lot of small tanks – is this about a yes-know answer or a how much answer?*

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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? I.e does disinfection work, even when mucus is present?

1. Collect a pool of infected mucus (as done previously by injecting a large number of abalone with a stock preparation of the herpesvirus). *Or go to a common frozen pool if any still left? May be more point in using fresh mucus for this trial as the physical characteristics may be more important.*

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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. *Drying, and the completeness and temperature of this, may be one of the most critical parts of the experiment, as drying is a major part of farm efforts to prevent re-infection. Do you need more effort here? If will stand prolonged drying, the relative importance of disinfection and drying, and the down-time of destocked tanks, may change considerably.*

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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. *While may want to inoculate some animals, it would also be good science to expose animals to the treated water as this is actually the type of exposure expected?*

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5. Maintain the experiment for 3 weeks during which time all mortalities will be recorded, and moribund abalone will be processed for histopathological examination. *3 weeks could be needed for in-contact low level exposure, but good indications should be present after a much shorter period by injection?*

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.

Ann: In short, more a matter of ensuring that the objective is clear, and that the number of animals used is within the capability of the resources available – often can't speed handling much just by having more hands available. Revisiting the objectives may see a change in emphasis in how this is done, but does not alter the desirability of knowing how well the virus survives in the environment and treatment with disinfectants, assuming that mucus may provide some protection and maintain foci of concentrated virus.

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.

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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

44

From: Annette Lyons [Annette@frdc.com.au]
Sent: Wednesday, 17 May 2006 10:54 AM
To: malcolm.lancaster@dpi.vic.gov.au
Cc: Crane, Mark (LI, Geelong); [s22]
Subject: re virus application
Attachments: FRDC out of cycle app 17 May 2006.doc

DELETION

Follow Up Flag: Follow up
Flag Status: Completed

Dear all

Attached is a draft of some of the sections of the application. Please feel free to alter anything you don't think is correct, necessary, etc.

Malcolm – could you work on the methodology, addressing Judith's and Mark's comments.

Mark G – Mike Wing rang yesterday to say that the director of Fisheries Wes Ford declined to assist financially. I haven't heard back from WA. Malcolm needs to know what the budget is before he can finalise methodology. So assuming we have failed to get state assistance, can you let Malcolm know what the budget is with AAGA contribution, etc.

Regards
Ann

Annette Lyons
Fisheries Research & Development Corporation

Email: annette.lyons@frdc.com.au
Telephone: (02) 6285 0417
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Development of cost effective and practical disinfection procedures for the eradication of the abalone herpes-like virus

BACKGROUND

Current status of the industry

Currently the Australia Aquaculture industry is a newly emerging industry producing approximately 300 tonne in 2004/05, worth $15 million. There are now 17 land based farms (SA: 5; Vic: 5; TAS: 5; WA:2), 5 seabased farms (SA: 3; Vic: 1; Tas: 1) and 17 hatcheries (SA: 6; Vic: 6; Tas: 4; WA: 2). The industry is projected to increase production by 20% annually as farms reach their full commercial output. Based on these figures, national production is predicted to reach approximately 750 tonne in 2009/10, worth $37.3 million.

Outline of the virus outbreak on farm

In December 2005 the first outbreak of a herpes-like virus occurred on two abalone farms in Victoria; Southern Ocean Mariculture (SOM) near Port Fairy (which produced 70 tonne in 2004/05) and Coastal Seafarms (CS) near Portland (which produced 50 tonne in 2004/05). Coastal Seafarms suffered losses of about 30% of their stock while the losses at SOM were less severe. Initial attempts to control the virus by partial de-stocking and biosecurity measures on farms were followed by a reduction in new cases. However, in February 2006 a second outbreak occurred on both farms and the losses were so severe, both companies decided to destock their entire farms. In addition, SOM lost all stock produced over the past six years as part of the industry's selective breeding program. The second outbreak at SOM appears to have been due to an infection via the inlet water as none of the biosecurity measures implemented after the first outbreak prevented transmission to new growout areas. In addition, stocks in the bay at the inlet were observed to be affected.

Comment [AEF1]:

Comment [AEF2]:

What is known about the abalone herpes-like viruses

Herpes virus neuritis is a newly recognised acute viral disease with high mortality that threatens both farmed and wild abalone industries in Australia. Initial transmission experiments conducted by Australian Animal Health Laboratories demonstrated that the virus was highly virulent with a short pre-clinical phase following experimental challenge, was transmitted via water, and survived freezing. Current work also includes a study of the effect of dilution on infectivity (that is, assessment of the minimum infective dose) and an on-farm study for indications of whether recurrence and persistence is due to re-infection from incoming water (implying possible external reservoir or infection), or to spread from within the farm.

A herpes-like virus has also been reported in China and Taiwan where massive losses of abalone stocks were suffered in the late 1990s. Only very basic work using light microscopy and EM has been published on this virus and poor reporting of sample sources and descriptions of what was being measured limited the value of these papers. Although not known formally, it is unlikely that either China or Taiwan is working on detection tools for the virus, such as PCR primers.

A "Disease Card" is being developed by the South East Asian Office for the International Control of Epizooitics (OIE) for the Chinese/Taiwan abalone virus, which is merely a compilation of known information on new and emerging diseases. It is likely that the abalone viral disease will move to a "listed disease" status in the near future. Once on this list the disease is reportable and there would be a full diagnostic chapter in the OIE Aquatic Diagnostic Manual.

NEED

In order to introduce practical control measures for the herpes-like virus it is necessary to confirm that the virus is transmitted by mucus and to identify an effective treatment for disinfecting farm equipment prior to restocking.

Studies of virus resistance will be directed to excreted mucus. The main body excretions of live animals consist of faeces, urine and mucus. As the known virus related lesions are in nervous tissue, there is no reason to suspect faeces would be a specific source of excretion. Urine and any direct haemolymph loss (and this would mostly be mucus associated) would readily be subject to dilution factors. Mucus is likely, therefore, to be the most likely source of concentrated virus in water outflows and tank surfaces, and by its nature may further protect the virus from degradation by environmental factors such as drying, and by disinfectants. Mucus is therefore the most suitable excretory product to evaluate virus degradation and disinfection.

The short term needs of those affected farms that have destocked are:

- To confirm that the virus is transmitted via mucus and

- To develop effective treatments for disinfecting farm equipment focusing on cost effectiveness, and minimum time of exposure to the treatment.

  As drying is likely to be the most cost effective method of disinfection on farms that have destocked and therefore have time for extended drying periods, this should be the focus of disinfection methods for these farms. For possible future outbreaks where affected farms might attempt to control the spread of the virus within the farm, disinfection methods need to be rapid. Similarly, routine biosecurity procedures are likely to require rapid disinfection of equipment that is used regularly, possibly a number of times a day. Thus a method that meets these criteria should also be developed (possibly using freshwater and/or drying and/or detergents and/or disinfectants).

BENEFIT

The abalone herpes virus threatens both farmed and wild abalone industries in Australia. It is critical that research is conducted on a number of fronts to address control and surveillance needs for a sound disease management strategy. This application addresses the immediate needs of abalone farmers currently affected by the virus in that it will deliver a reliable method of disinfecting their farm before restocking. It also will provide a disinfection protocol for possible future viral outbreaks where farmers will no doubt attempt to control the spread of the virus within the farm through strict quarantine and biosecurity procedures.

As a consequence of the viral outbreak it is anticipated that many, if not all, farms will fast track their recent efforts to implement health surveillance programs within each state and to introduce routine biosecurity practises for all staff on-farm. It is likely that a biosecurity protocol would include routine disinfection of tools, boots, equipment, tanks, etc. This proposed work delivers the necessary disinfection procedure for such a protocol.

INDUSTRY CONSULTATION

The FRDC's leaders of the Aquatic Animal Health Subprogram and the Abalone Aquaculture Subprogram, Drs Mark Crane and Ann Fleming, have assisted the managers of the affected farms and Dr Malcolm Lancaster of the Department of PI, Victoria to develop this research proposal. A meeting was held on one of the farms

during the viral outbreak to discuss research needs and to allow Drs Crane and Lancaster to see the farm situation first hand. Dr Judith Handlinger provided input into clarifying objectives and commented on methodology. Subsequently, the farm managers were asked to provide further refinements to the objectives.

Dr Fleming has kept the Australian Abalone Growers Association informed of the development of this proposal and has begun work with the association to identify future research needed to develop sound management strategies for the virus.

RELATED WORK

Australian Animal Health Laboratories research

The preliminary work conducted by Dr Mark Crane at the Australian Animal Health Laboratories after the first outbreak of the virus in December 2005 is the most relevant work to this proposal. The following is a summary of the work done and available results to date:

1) The first series of trials aimed to determine how virulent was the transfer of the virus, can it pass through the water column and can freezing kill it.

   - Infected tissue was inoculated into healthy animals. Sick in 2-3 days. Thus virus very virulent.

   - Sick abalone were held in a basket suspended in an aquarium with healthy animals on the floor below. Healthy abalone were sick in 4-5 days. Thus vector is through the water column.

   - Frozen tissue was inoculated into healthy abalone. Sick in 3-4 days. Thus virus can survive freezing.

2) There were concern from the Department of PI, Victoria that the virus might travel through the effluent and contaminate wild abalone and other species. The following experiment was set up to determine the minimum level of dilution required to ensure no infection occurs.

- Water was taken from the farm and diluted down at four dilutions from 100% to 0.001% (or something similar – not sure of the exact dilutions). Healthy abs are placed in each dilution and monitored for signs of virus. Water was also taken from a non-infected area and contaminated with sick abs and tested in the same manner. Results are not available yet.

Comment [AEF3]: Can we get these results now Mark C.?

3) Assess available PCR primers for their suitability to detect the virus in abalone.

- A PCR-primer was accessed from colleagues in France working on an oyster virus. So far Dr Crane has tested a handful of gene sequences from the abalone virus for their reactivity with the primer, but so far there has been no positive results.

Southern Ocean Mariculture research

Comment [AEF4]: Mark G – Do you have any preliminary results yet?

Mark Gervis of Southern Ocean Mariculture is currently conducting research to investigate whether the virus is originating from the inflow water at Southern Ocean Mariculture and Coastal Seafarms.

The experiment is set up in isolated rooms at each farm to keep it completely quarantined from the rest of the farm. The tanks and the equipment were disinfected with Virkon S at a rate of 1:100. At SOM the experiment is set up in the spawning room and at CS in the brood stock conditioning room. Both rooms have not been used for at least two months and they had been fully disinfected and dried out.

Abalone (n= 100) of approximately 30mm shell length were used by each farm as this age/size class has been most susceptible to the virus. The animals are fed five times a week and cleaned twice a week. Every effort was made to 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 is assigned to the area. All feeding, cleaning and general maintenance is 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 for confirmation of the virus.

Gribbles Pathology research

Dr David Tisdall is a virologist based at Gribbles, Adelaide and is responsible for R&D for the company. After the first viral outbreak he began a preliminary assessment of a PCR-primer for an oyster virus for its reactivity with the abalone virus. To date he has not had any positive results but is now testing alternative primers.

45

From: Crane, Mark (LI, Geelong)
Sent: Monday, 22 May 2006 10:48 AM
To: Corbeil, Serge (LI, Geelong)
Cc: Walker, Peter (LI, Geelong)
Subject: Abalone budget

Follow Up Flag: Follow up
Flag Status: Completed

Serge,

Apologies for the the confusion re: budget. I have corrected the mistake (see attachment). Also, it is likely that you will be doing other work apart from Abalone herpesvirus and so your time should probably be less than 100% on this project. Is there any technical assistance required - could Nette be involved and learn some molecular biology?

Mark

research costing
abs herpes 06...

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su7P

version 7/2/06			2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
			0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
FUNDING ORGANISATION		0													
START DATE		0													
PROJECT		0													
Site		Geelong													
			2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
			0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
SALARIES															
NAME			SALARY		SALARY		SALARY		SALARY		SALARY			SALARY	
	☑		0	0	0	39149	0	81429	0	44915	0	0		0	165493
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	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
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	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
DIRECT COSTS															
salary			0	0	0	39149	0	81429	0	44915	0	0		0	165493
oncosts			0	0	0	9787	0	20357	0	11229	0	0		0	41373
total salary			0	0	0	48936	0	101787	0	56143	0	0		0	206866
TOTAL STAFF			0.00		0.50		1.00		0.50		0.00			2.00	
travel	☑		0	0	0	0	0	1000	0	1000	0	0		0	2000
materials	☑		0	0	0	10000	0	15000	0	10000	0	0		0	35000
other ie PCR, DNAseq etc	☐		0	0	0	0	0	0	0	0	0	0		0	0
animals	☐		0	0	0	0	0	0	0	0	0	0		0	0
TOTAL DIRECT COSTS			0	0	0	58936	0	117787	0	67143	0	0		0	243866
DIRECT OVERHEADS															
Photography ($40 per hour)	☑		0	0	0	0	0	200	0	200	0	0		0	400
Animal Services	☑		0	0	0	0	0	0	0	0	0	0		0	0
Engineering & Site Services	☑		0	0	0	0	0	0	0	0	0	0		0	0
Scientific services	☐		0	0	0	0	0	0	0	0	0	0		0	0
Depreciation equipment	☐		0	0	3572	0	7430	0	4098	0	0	0		15101	0
TOTAL DIRECT OVERHEADS			0	0	3572	0	7430	200	4098	200	0	0		15101	400
INDIRECT OVERHEADS ☐															
Executive management	☐		0	0	4894	0	10179	0	5614	0	0	0		20687	0
Finance	☐		0	0	1796	0	3736	0	2060	0	0	0		7592	0
Human Resources	☐		0	0	1365	0	2840	0	1566	0	0	0		5772	0
OH&S	☐		0	0	891	0	1853	0	1022	0	0	0		3765	0
Computing	☐		0	0	1551	0	3227	0	1780	0	0	0		6558	0
Commercial operations	☐		0	0	2178	0	4530	0	2498	0	0	0		9206	0
Communications	☐		0	0	940	0	1954	0	1078	0	0	0		3972	0
Records Management	☐		0	0	621	0	1293	0	713	0	0	0		2627	0
Library	☐		0	0	1571	0	3267	0	1802	0	0	0		6640	0
Security	☐		0	0	568	0	1181	0	651	0	0	0		2400	0
Micro security	☐		0	0	0	0	0	0	0	0	0	0		0	0
Quality Assurance	☐		0	0	0	0	0	0	0	0	0	0		0	0
Lab services	☐		0	0	377	0	784	0	432	0	0	0		1593	0
Animal Services- availability	☐		0	0	3719	0	7736	0	4267	0	0	0		15722	0
Store	☐		0	0	1752	0	3644	0	2010	0	0	0		7406	0
Maintenance/Site Services	☐		0	0	4962	0	10321	0	5693	0	0	0		20976	0
Utilities	☐		0	0	915	0	1903	0	1050	0	0	0		3868	0
Animal Ethics	☐		0	0	411	0	855	0	472	0	0	0		1738	0
Other(incl photo/reception/overheads)	☐		0	0	274	0	570	0	314	0	0	0		1158	0
Depreciation AAHL Building	☐		0	0	4404	0	9161	0	5053	0	0	0		18618	0
TOTAL INDIRECT OVERHEADS			0	0	33188	0	69032	0	38076	0	0	0	0	140296	0
			0		0		0		0		0			0	0
CORPORATE OVERHEADS	☐		0	0	5383	0	11197	0	6176	0	0	0		22755	0
			0		0		0		0		0			0	0
TOTAL COSTS			0	0	42144	58936	87659	117987	48351	67343	0	0		178153	244266
EXTERNAL FUNDING			0		65000		125000		72000		0			262000	

version 7/2/06

	NAME	2005/06 CSOF	%	2006/07 CSOF	%	2007/08 CSOF	%	2008/09 CSOF	%	2009/10 CSOF	%
emplyee 1	Serge Corbeil	1.1	0%	5M	50%	5M	100%	6.1	50%	6.2	0%
emplyee 2		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 3		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 4		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 5		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 6		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 7		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 8		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 9		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 10		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 11		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 12		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 13		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 14		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplyee 15		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%

Rocky, Armidale, Floreat Pa

	YEAR 1 rate per day	no. of days	no. of anima total	YEAR 2 rate per day	no. of days	no. of anin total	YEAR 3 rate per day	no. of days	no. of ani total	YEAR 4 rate per da	no. of	no. of anin total	YEAR 5 rate per da	no. of days	no. of anin total
cattle	4.3		0	4.3		0	4.3		0	4.3		0	4.3		0
sheep,goats,pigs	0.86		0	0.86		0	0.86		0	0.86		0	0.86		0
poultry	0.43		0	0.43		0	0.43		0	0.43		0	0.43		0
other	0		0	0		0	0		0	0		0	0		0
			0			0			0			0			0

Belmont, Yalanbee, Chiswic

	YEAR 1 rate per hour	no. of hours	total	YEAR 2 rate per hour	no. of hours	total	YEAR 3 rate per hour	no. of hours	total	YEAR 4 rate per hc	no. of hours	total	YEAR 5 rate per hc	no. of hours	total
	45		0	**47**		0	**49**		0	**51**		0	**53**		0

Large Animal Facility, Geelo

	YEAR 1 Rate per day	No.of days	No.of rooms total	YEAR 2 Rate per day	No.of days	No.of roon total	YEAR 3 Rate per day	No.of days	No.of roc total	YEAR 4 Rate per d	No.of	No.of roon total	YEAR 5 Rate per d	No.of days	No.of roon total
	157		0	**164**		0	**172**		0	**181**		0	**190**		0

Small Animal Facility, Geelo

	YEAR 1 rate per box per day	no. of days	No. of boxes total	YEAR 2 rate per box pe	no. of days	No. of box total	YEAR 3 rate per box p	no. of days	No. of bo total	YEAR 4 rate per bc	no. of	No. of box total	YEAR 5 rate per bc	no. of days	No. of box total
Rabbits	16.00		0	16.80		0	17.64		0	18.52		0	19.45		0
Poultry	20.00		0	21.00		0	22.05		0	23.15		0	24.31		0
Poultry SPF	20.00		0	21.00		0	22.05		0	23.15		0	24.31		0
Rats	4.00		0	4.20		0	4.41		0	4.63		0	4.86		0
Mice	3.00		0	3.15		0	3.31		0	3.47		0	3.65		0
			0			0			0			0			0

Werribee

Paddock	$22 per day
Building 5 - small rooms	$94 per day
Building 5, 7 or 8	$188 per day

Animal accomodation	YEAR 1 No. of rooms	rate per day	No. of days	total	YEAR 2 No. of rooms	rate per da	No. of day	total	YEAR 3 No. of rooms	rate per da	No. of da	total	YEAR 4 lo. of room	rate per	No. of day	total	YEAR 5 lo. of room	rate per da	No. of day	total
Paddock		22		0		22		0		22		0		22		0		22		0
Building 5 - small		94		0		94		0		94		0		94		0		94		0
Buidling 5, 7 or 8		188		0		188		0		188		0		188		0		188		0
				0				0				0				0				0

DAYS CONVERTER

DAYS	%
2	0.0091
HOURS	
0	0.0000

A.1	19202	Salary scales current as at 13/11/05
A.2	24001	
A.3	28802	
AM	34562	
1.1	28667	
1.2	29399	
1.3	30130	
1.4	30862	
1.5	31594	
1M	32325	
2.1	33554	
2.2	34766	
2.3	35977	
2.4	37189	
2.5	38402	
2.6	39614	
2.7	40827	
2.8	42039	
2M	43250	
3.1	44808	
3.2	46354	
3.3	47878	
3.4	49403	
3.5	50929	
3.6	52454	
3.7	53980	
3.8	55504	
3M	57028	
4.1	58965	
4.2	60901	
4.3	62838	
4.4	64774	
4M	66711	
5.1	69569	
5.2	72428	
5M	75286	
6.1	79858	
6.2	84431	
6.3	89004	
6M	93578	
7.1	98134	
7.2	98691	
7.3	101247	
7.4	103803	
7M	106363	
8.1	114709	
8.2	123055	
8.3	133538	
8M	144020	
9.1	150367	
9.2	156713	
9.3	163060	
9M	169404	

version 7/2/06

PRICING DECISION STATEMENT

CONTRACT:
CUSTOMER:
PROJECT: *0*

Type of Activity
- ○ Consulting or Technical Service
- ○ Research - Contract
- ◉ Research - Collaborative - subject to CN
- ○ Research - Collaborative - NOT subject to CN

COSTS:	Direct Costs (less Costs of Sub-contracts)	**259,367**	
	Indirect Costs	**163,052**	
	Cost of Sub-Contracted Activities	-	
	Full-cost Price	**422,419**	
	Competitive Neutrality	64,208	
	Full-cost Price plus competitive neutrality	486,627	
PRICE:	**Cash Contribution**	**262,000**	101% DIRECT COSTS ; 62% TOTAL COSTS
	CSIRO - Livestock Industries In-kind Contributions	**160,419**	
	Value of Other Considerations (1)		

Justification (if applicable) (2):

Prepared by: _____ **Recommended by:** www
Project Leader — Commercial Manager

Approved by: _____ **Date:** _____
Delegate

NOTES:

(1) Other forms of Consideration include long term value of licence fees and royalties

(2) Justifications for prices lower than Full-cost Price include National Interest (specify it) and commercial considerations

547F

version 7/2/06		2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
		0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
FUNDING ORGANISATION	0													
START DATE	0													
PROJECT	0													
Site	Geelong													
		2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
		0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
SALARIES														
NAME		SALARY		SALARY		SALARY		SALARY		SALARY			SALARY	
		0	0	0	39149	0	81429	0	44915	0	0		0	165493
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
0		0	0	0	0	0	0	0	0	0	0		0	0
DIRECT COSTS														
salary		0	0	0	39149	0	81429	0	44915	0	0		0	165493
oncosts		0	0	0	9787	0	20357	0	11229	0	0		0	41373
total salary		0	0	0	48936	0	101787	0	56143	0	0		0	206866
TOTAL STAFF		0.00		0.50		1.00		0.50		0.00			2.00	
travel		0	0	0	0	0	1000	0	1000	0	0		0	2000
materials		0	0	0	10000	0	15000	0	10000	0	0		0	35000
other ie PCR, DNAseq etc		0	0	0	0	0	0	0	0	0	0		0	0
animals		0	0	0	0	0	0	0	0	0	0		0	0
TOTAL DIRECT COSTS		0	0	0	58936	0	117787	0	67143	0	0		0	243866
DIRECT OVERHEADS														
Photography ($40 per hour)		0	0	0	0	0	200	0	200	0	0		0	400
Animal Services		0	0	0	0	0	0	0	0	0	0		0	0
Engineering & Site Services		0	0	0	0	0	0	0	0	0	0		0	0
Scientific services		0	0	0	0	0	0	0	0	0	0		0	0
Depreciation equipment		0	0	3572	0	7430	0	4098	0	0	0		15101	0
TOTAL DIRECT OVERHEADS		0	0	3572	0	7430	200	4098	200	0	0		15101	400
INDIRECT OVERHEADS														
Executive management		0	0	4894	0	10179	0	5614	0	0	0		20687	0
Finance		0	0	1796	0	3736	0	2060	0	0	0		7592	0
Human Resources		0	0	1365	0	2840	0	1566	0	0	0		5772	0
OH&S		0	0	891	0	1853	0	1022	0	0	0		3765	0
Computing		0	0	1551	0	3227	0	1780	0	0	0		6558	0
Commercial operations		0	0	2178	0	4530	0	2498	0	0	0		9206	0
Communications		0	0	940	0	1954	0	1078	0	0	0		3972	0
Records Management		0	0	621	0	1293	0	713	0	0	0		2627	0
Library		0	0	1571	0	3267	0	1802	0	0	0		6640	0
Security		0	0	568	0	1181	0	651	0	0	0		2400	0
Micro security		0	0	0	0	0	0	0	0	0	0		0	0
Quality Assurance		0	0	0	0	0	0	0	0	0	0		0	0
Lab services		0	0	377	0	784	0	432	0	0	0		1593	0
Animal Services- availability		0	0	3719	0	7736	0	4267	0	0	0		15722	0
Store		0	0	1752	0	3644	0	2010	0	0	0		7406	0
Maintenance/Site Services		0	0	4962	0	10321	0	5693	0	0	0		20976	0
Utilities		0	0	915	0	1903	0	1050	0	0	0		3868	0
Animal Ethics		0	0	411	0	855	0	472	0	0	0		1738	0
Other(incl photo/reception/overheads)		0	0	274	0	570	0	314	0	0	0		1158	0
Depreciation AAHL Building		0	0	4404	0	9161	0	5053	0	0	0		18618	0
TOTAL INDIRECT OVERHEADS		0	0	33188	0	69032	0	38076	0	0	0	0	140296	0
		0		0		0		0		0			0	0
CORPORATE OVERHEADS		0	0	5383	0	11197	0	6176	0	0	0		22755	0
		0		0		0		0		0			0	0
TOTAL COSTS		0	0	42144	58936	87659	117987	48351	67343	0	0		178153	244266
EXTERNAL FUNDING		0		65000		125000		72000		0			262000	

From: Crane, Mark (LI, Geelong)
Sent: Tuesday, 23 May 2006 3:33 PM
To: Corbeil, Serge (LI, Geelong)
Subject: Latest versions for Abalone preproposal

Follow Up Flag: Follow up
Flag Status: Completed

Serge,

As discussed yesterday, Peter is happy for us to submit this preproposal. I have added the DPI Vic components (Malcolm will need to add in-kind contributions). Please review and accept/reject changes as needed and then we can send it up to Malcolm for his review and comment.

Mark


FRDC Abalone herpesvirus 06052..


research costing abs herpes 06...

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

International Phone: +61 3 52 275118
International Fax: +61 3 52 275555
email: mark.crane@csiro.au

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: Development and application of Mmolecular characterisationdiagnostic procedures for of an emerging abalone herpes-like virus

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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: 310-016-2009

Preliminary Budget *(insert $$s)*

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FRDC Contribution	2006-07	2007-08	2008-09	2009-10	TOTAL $$s
Salaries and on costs (AAHL)	39 149	81 429	44 915		165 493
Salaries and on costs (DPI Vic – PhD student)		25 000	25 000	25 000	75 000
Travel		1 000	1 000	1000	3 000
Operating	12 858	31 144	23 479	10 000	77 481
Capital (equipment)					
FRDC TOTAL	**52 007**	**103 573**	**59 394**		**320 974**
Research Organisation contribution (AAHL)	30 857	64 183	35 401		130 441
Research Organisation contribution (DPI Vic)					
Total of Industry & Other Funding	x	x	x	x	x
GRAND TOTAL in $$s					

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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. Detection of low levels of virus in asymptomatic carriers (e.g. wild abalone broodstock and/or other aquatic animal species) requires the development of these highly specific and sensitive molecular reagents. The use of these molecular tools will also facilitate a better understanding of the epidemiology of this disease, leading to more efficient management of disease outbreaks.

In addition to surveillance tools, better procedures/reagents for disease diagnosis are required. Presence of histological lesions provides a presumptive diagnosis. The development of in situ hybridisation probe(s) and diagnostic antiserum for the localisation of abalone herpesvirus within histological lesions will provide a means for definitive diagnosis to be made.

Objectives

1. To characteriseidentify nucleic acid sequences of the emerging abalone herpesvirus at the molecular level (via PCR-based gene amplification and sequencing) useful for the development of diagnostic tools (e.g. conventional PCR, TaqMan PCR, ISH probe).
2. To develop and validate a real-time TaqMan PCR assay for the detection of the abalone virus.
3. To develop an in situ hybridisation probe specific for the abalone herpesvirus
4. To determine which abalone tissues provide accurate diagnosis of infection.
3.5. To develop an diagnostic antiserum for use as an immunohistochemical reagent in diagnosis of herpesvirus infections.
4.6. To document an Australian and New Zealand Standard Diagnostic Procedure and submit for external review.

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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 tests 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.

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3. A further output from this project will be a newly qualified diagnostic virologist trained in modern diagnostic techniques that would be suitable for a diagnostic/research position in a State laboratory or research institution.

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Estimated Flow of Benefits

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Fisheries and aquaculture managed by States/Territories	
NSW	5%
Qld	1%
SA	15%
Tas	49%
Vic	25%
WA	5%
AFMA managed fisheries	0%

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Other beneficiaries	0%
Total for all fisheries	100%

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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.
-Herpesvirus nucleic acid sequences suitable for use as a diagnostic probe will be developed for in situ hybridisation.
-Using the Primer Express SofwareSoftware 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 underwithin the AAHL biosecuritye facility, to determine which sampling and testing of various abalone tissues are the most appropriate for sampling during an active surveillance program of, for example, wild broodstock.
-Either virus will be purified from host tissue or virus proteins will be engineered to provide antigens for production of diagnostic polyclonal antiserum in rabbits 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
CSIRO Livestock Industries
Private Bag 24, Geelong VIC 3220

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Phone: 03 5227 5427
Fax: 02 5227 5555
E-MAIL: joanne.slater@csiro.au
Website: http://www.frdc.com.au/research/programs/aah/index.htm

No later than cob on Friday 23 June 2006.

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FUNDING ORGANISATION							
START DATE							
PROJECT							
Site		Geelong					
		2005/06	2006/07	2007/08	2008/09	2009/10	TOTAL
SALARIES							
NAME	LEVEL	SALARY	SALARY	SALARY	SALARY	SALARY	SALARY
		0	31319	65143	35932	0	132394
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
0		0	0	0	0	0	0
DIRECT COSTS							
salary		0	31319	65143	35932	0	132394
onc[illegible]		0	7830	16286	8983	0	33099
total salary		0	39149	81429	44915	0	165493
TO[illegible] STAFF		0.00	0.40	0.80	0.40	0.00	
travel				1000	1000		2000
materials			10000	15000	10000	0	35000
other ie PCR, DNAseq etc							0
animals							0
TOTAL DIRECT COSTS		0	49149	97429	55915	0	202493
DIRECT OVERHEADS							
Photography ($50 per hour)				200	200		400
Animal Services		0	0	0	0	0	0
Engineering & Site Services							0
Scientific services							
Depreciation equipment		0	2858	5944	3279	0	12081
TOTAL DIRECT OVERHEADS		0	2858	6144	3479	0	12481
INDIRECT OVERHEADS							
Executive management		0	3915	8143	4491	0	16549
Finance		0	1437	2988	1648	0	6074
People Development		0	1092	2272	1253	0	4617
OH&S		0	713	1482	817	0	3012
Computing		0	1241	2581	1424	0	5246
Commercial operations		0	1742	3624	1999	0	7364
Marketing/Communications		0	752	1563	862	0	3177
Records Management		0	497	1034	570	0	2102
Library		0	1257	2614	1442	0	5312
Se[illegible]		0	454	945	521	0	1920
Mic[illegible]curity		0	0	0	0	0	0
Quality Assurance		0	0	0	0	0	0
Lab services		0	301	627	346	0	1274
Animal Services- availability		0	2975	6189	3414	0	12577
Store		0	1402	2915	1608	0	5925
Maintenance/Site Services		0	3970	8257	4554	0	16781
Utilities		0	732	1523	840	0	3095
Animal Ethics		0	329	684	377	0	1390
Other(incl photo/reception/overheads)		0	219	456	252	0	927
Depreciation AAHL Building		0	3523	7329	4042	0	14894
TOTAL INDIRECT OVERHEADS		0	26551	55225	30461	0	112237
CORPORATE OVERHEADS		0	4306	8957	4941	0	18204
TOTAL COSTS		**0**	**82864**	**167756**	**94795**	**0**	**345415**
COMPETITIVE NEUTRALITY ONCOST FACTOR		**0**	**12595**	**25499**	**14409**	**0**	**52503**
EXTERNAL FUNDING			**52007**	103573	**59394**	**0**	**214974**
EXTERNAL FUNDING % TOTAL COSTS		#DIV/0!	63%	62%	63%	#DIV/0!	62%
IN-KIND CONTRIBUTIONS		**0**	**30857**	**64183**	**35401**	**0**	**130441**
MULTIPLIER		**#DIV/0!**	**2.65**	**2.58**	**2.64**	**#DIV/0!**	**2.61**
SIGNED		SIGNED				SIGNED	
PROJECT LEADER		COMMERCIAL MANAGER				DELEGATE	

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NAME	2005/06 CSOF	%	2006/07 CSOF	%	2007/08 CSOF	%	2008/09 CSOF	%	2009/10 CSOF	%
emplvee 1	Serge Corbeil	1.1	0%	5M	40%	5M	80%	6.1	40%	6.2	0%
emplvee 2		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 3		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 4		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 5		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 6		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 7		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 8		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 9		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 10		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 11		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 12		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 13		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 14		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%
emplvee 15		1.1	0%	1.1	0%	1.1	0%	1.1	0%	1.1	0%

Rocky, Armidale, Floreat Pa

	YEAR 1 rate per day	no. of days	no. of anima total	YEAR 2 rate per day	no. of days	no. of anin total	YEAR 3 rate per day	no. of days	no. of ani total	YEAR 4 rate per da	no. of	no. of anin total	YEAR 5 rate per da	no. of days	no. of anin total
cattle	4.3		0	4.3		0	4.3		0	4.3		0	4.3		0
sheep,goats,pigs	0.86		0	0.86		0	0.86		0	0.86		0	0.86		0
poultry	0.43		0	0.43		0	0.43		0	0.43		0	0.43		0
other	0		0	0		0	0		0	0		0	0		0
			0			0			0			0			0

Belmont, Yalanbee, Chiswic

	YEAR 1 rate per hour	no. of hours	total	YEAR 2 rate per hour	no. of hours	total	YEAR 3 rate per hour	no. of hours	total	YEAR 4 rate per hc	no. of hours	total	YEAR 5 rate per hc	no. of hours	total
	45		0	47		0	49		0	51		0	53		0

Large Animal Facility, Geelo

	YEAR 1 Rate per day	No.of days	No.of rooms total	YEAR 2 Rate per day	No.of days	No.of roon total	YEAR 3 Rate per day	No.of days	No.of roc total	YEAR 4 Rate per d	No.of	No.of roon total	YEAR 5 Rate per d	No.of days	No.of roon total
	157		0	164		0	172		0	181		0	190		0

Small Animal Facility, Geelo

	YEAR 1 rate per box per day	no. of days	No. of boxes total	YEAR 2 rate per box pe	no. of days	No. of box total	YEAR 3 rate per box p	no. of days	No. of bo total	YEAR 4 rate per bc	no. of	No. of box total	YEAR 5 rate per bc	no. of days	No. of box total
Rabbits	16.00		0	16.80		0	17.64		0	18.52		0	19.45		0
Poultry	20.00		0	21.00		0	22.05		0	23.15		0	24.31		0
Poultry SPF	20.00		0	21.00		0	22.05		0	23.15		0	24.31		0
Rats	4.00		0	4.20		0	4.41		0	4.63		0	4.86		0
Mice	3.00		0	3.15		0	3.31		0	3.47		0	3.65		0
			0			0			0			0			0

Werribee

Paddock	$22 per day
Building 5 - small rooms	$94 per day
Building 5, 7 or 8	$188 per day

Animal accomodation	YEAR 1 No. of rooms	rate per day	No. of days	total	YEAR 2 No. of rooms	rate per da	No. of day	total	YEAR 3 No. of rooms	rate per da	No. of da	total	YEAR 4 lo. of room	rate pe	No. of day	total	YEAR 5 lo. of room	rate per da	No. of day	total
Paddock		22		0		22		0		22		0		22		0		22		0
Building 5 - small		94		0		94		0		94		0		94		0		94		0
Buidling 5, 7 or 8		188		0		188		0		188		0		188		0		188		0
				0				0				0				0				0

DAYS CONVERTER

DAYS	%
2	0.0091
HOURS	
0	0.0000

A.1	19202	Salary scales current as at 13/11/05
A.2	24001	
A.3	28802	
AM	34562	
1.1	28667	
1.2	29399	
1.3	30130	
1.4	30862	
1.5	31594	
1M	32325	
2.1	33554	
2.2	34766	
2.3	35977	
2.4	37189	
2.5	38402	
2.6	39614	
2.7	40827	
2.8	42039	
2M	43250	
3.1	44808	
3.2	46354	
3.3	47878	
3.4	49403	
3.5	50929	
3.6	52454	
3.7	53980	
3.8	55504	
3M	57028	
4.1	58965	
4.2	60901	
4.3	62838	
4.4	64774	
4M	66711	
5.1	69569	
5.2	72428	
5M	75286	
6.1	79858	
6.2	84431	
6.3	89004	
6M	93578	
7.1	98134	
7.2	98691	
7.3	101247	
7.4	103803	
7M	106363	
8.1	114709	
8.2	123055	
8.3	133538	
8M	144020	
9.1	150367	
9.2	156713	
9.3	163060	
9M	169404	

version 7/2/06

PRICING DECISION STATEMENT

CONTRACT:
CUSTOMER:
PROJECT: *0*

Type of Activity
- ○ Consulting or Technical Service
- ○ Research - Contract
- ◉ Research - Collaborative - subject to CN
- ○ Research - Collaborative - NOT subject to CN

COSTS:	Direct Costs (less Costs of Sub-contracts)	**214,974**		
	Indirect Costs	**130,441**		
	Cost of Sub-Contracted Activities	-		
	Full-cost Price	**345,415**		
	Competitive Neutrality	52,503		
	Full-cost Price plus competitive neutrality	397,918		
PRICE:	**Cash Contribution**	**214,974**	100% DIRECT COSTS ;	62% TOTAL COSTS
	CSIRO - Livestock Industries In-kind Contributions	**130,441**		
	Value of Other Considerations (1)			

Justification (if applicable) (2):

Prepared by: _____ **Recommended by:** www
Project Leader — Commercial Manager

Approved by: _____ **Date:** _____
Delegate

NOTES:

(1) Other forms of Consideration include long term value of licence fees and royalties

(2) Justifications for prices lower than Full-cost Price include National Interest (specify it) and commercial considerations

541F

version 7/2/06			2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
			0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
FUNDING ORGANISATION		0													
START DATE		0													
PROJECT		0													
Site		Geelong													
			2005/06		2006/07		2007/08		2008/09		2009/10			TOTAL	
SALARIES			0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND	0	IN KIND		0	IN KIND
NAME			SALARY		SALARY		SALARY		SALARY		SALARY			SALARY	
	☑		0	0	0	31319	0	65143	0	35932	0	0		0	132394
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☐		0	0	0	0	0	0	0	0	0	0		0	0
	0 ☑		0	0	0	0	0	0	0	0	0	0		0	0
DIRECT COSTS															
salary			0	0	0	31319	0	65143	0	35932	0	0		0	132394
oncosts			0	0	0	7830	0	16286	0	8983	0	0		0	33099
total salary			0	0	0	39149	0	81429	0	44915	0	0		0	165493
TOTAL STAFF			0.00		0.40		0.80		0.40		0.00			1.60	
travel	☑		0	0	0	0	0	1000	0	1000	0	0		0	2000
materials	☑		0	0	0	10000	0	15000	0	10000	0	0		0	35000
other ie PCR, DNAseq etc	☐		0	0	0	0	0	0	0	0	0	0		0	0
animals	☐		0	0	0	0	0	0	0	0	0	0		0	0
TOTAL DIRECT COSTS			0	0	0	49149	0	97429	0	55915	0	0		0	202493
DIRECT OVERHEADS															
Photography ($40 per hour)	☑		0	0	0	0	0	200	0	200	0	0		0	400
Animal Services	☑		0	0	0	0	0	0	0	0	0	0		0	0
Engineering & Site Services	☑		0	0	0	0	0	0	0	0	0	0		0	0
Scientific services	☐		0	0	0	0	0	0	0	0	0	0		0	0
Depreciation equipment	☐		0	0	2858	0	5944	0	3279	0	0	0		12081	0
TOTAL DIRECT OVERHEADS			0	0	2858	0	5944	200	3279	200	0	0		12081	400
INDIRECT OVERHEADS ☐															
Executive management	☐		0	0	3915	0	8143	0	4491	0	0	0		16549	0
Finance	☐		0	0	1437	0	2988	0	1648	0	0	0		6074	0
Human Resources	☐		0	0	1092	0	2272	0	1253	0	0	0		4617	0
OH&S	☐		0	0	713	0	1482	0	817	0	0	0		3012	0
Computing	☐		0	0	1241	0	2581	0	1424	0	0	0		5246	0
Commercial operations	☐		0	0	1742	0	3624	0	1999	0	0	0		7364	0
Communications	☐		0	0	752	0	1563	0	862	0	0	0		3177	0
Records Management	☐		0	0	497	0	1034	0	570	0	0	0		2102	0
Library	☐		0	0	1257	0	2614	0	1442	0	0	0		5312	0
Security	☐		0	0	454	0	945	0	521	0	0	0		1920	0
Micro security	☐		0	0	0	0	0	0	0	0	0	0		0	0
Quality Assurance	☐		0	0	0	0	0	0	0	0	0	0		0	0
Lab services	☐		0	0	301	0	627	0	346	0	0	0		1274	0
Animal Services- availability	☐		0	0	2975	0	6189	0	3414	0	0	0		12577	0
Store	☐		0	0	1402	0	2915	0	1608	0	0	0		5925	0
Maintenance/Site Services	☐		0	0	3970	0	8257	0	4554	0	0	0		16781	0
Utilities	☐		0	0	732	0	1523	0	840	0	0	0		3095	0
Animal Ethics	☐		0	0	329	0	684	0	377	0	0	0		1390	0
Other(incl photo/reception/overheads)	☐		0	0	219	0	456	0	252	0	0	0		927	0
Depreciation AAHL Building	☐		0	0	3523	0	7329	0	4042	0	0	0		14894	0
TOTAL INDIRECT OVERHEADS			0	0	26551	0	55225	0	30461	0	0	0	0	112237	0
			0		0		0		0		0			0	0
CORPORATE OVERHEADS	☐		0	0	4306	0	8957	0	4941	0	0	0		18204	0
			0		0		0		0		0			0	0
TOTAL COSTS			0	0	33715	49149	70127	97629	38681	56115	0	0		142522	202893
EXTERNAL FUNDING			0		52007		103573		59394		0			214974	

47

From: Crane, Mark (LI, Geelong)
Sent: Wednesday, 24 May 2006 5:03 PM
To: Malcolm Lancaster (malcolm.lancaster@dpi.vic.gov.au)
Cc: Corbeil, Serge (LI, Geelong)
Subject: FRDC Preproposal

Follow Up Flag: Follow up
Flag Status: Completed

Malcolm,

As promised, here is the FRDC preproposal. Serge and I have put in the DPI Vic component (PhD salary and operating) including milestones etc. You will note that the PhD starts 6 months after the AAHL component starts so that we get some sequence prior to the student's start.

Please put DPI Vic's in-kind contribution (overhead costs and salaries of any student supervisors time etc) in the budget table for the appropriate years. Then I can finalise the proposal and send it to the FRDC Subprogram for evaluation.

Cheers

Mark

FRDC Abalone
herpesvirus 06052.

MARK CRANE Ph.D.
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International Fax: +61 3 52 275555
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AQUATIC ANIMAL HEALTH SUBPROGRAM

2007-08 Preliminary Research Proposal

Project Title

Aquatic Animal Health Subprogram: Development and application of molecular diagnostic procedures for 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: 30-06-2009

Preliminary Budget

FRDC Contribution	2006-07	2007-08	2008-09	2009-10	TOTAL $$s
Salaries and on costs (AAHL)	39 149	81 429	44 915		165 493
Salaries and on costs (DPI Vic – PhD student)		25 000	25 000	25 000	75 000
Travel		1 000	1 000	1000	3 000
Operating	12 858	31 144	23 479	10 000	77 481
Capital (equipment)					
FRDC TOTAL	**52 007**	**103 573**	**59 394**		**320 974**
Research Organisation contribution (AAHL)	30 857	64 183	35 401		130 441
Research Organisation contribution (DPI Vic)					
Total of Industry & Other Funding	x	x	x	x	x
GRAND TOTAL in $$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. Detection of low levels of virus in asymptomatic carriers (e.g. wild abalone broodstock and/or other aquatic animal species) requires the development of these highly specific and sensitive molecular tools such as a quantitative PCR assay.
In addition to surveillance tools, better procedures/reagents for disease diagnosis are required. Presence of histological lesions provides a presumptive diagnosis. The development of in situ hybridisation probe(s) and diagnostic antiserum for the localisation of abalone herpesvirus within histological lesions will provide a means for definitive diagnosis to be made. The development of molecular tools and reagents will allow researchers and industry to rapidly and specifically detect and locate the virus in abalone tissues therefore providing a vital means for diagnosis and facilitating a better understanding of the epidemiology of this disease, leading to more efficient management of disease outbreaks.

Objectives

1. To identify nucleic acid sequences of the emerging abalone herpesvirus (via PCR-based gene amplification and sequencing) necessary for the development of diagnostic tools (e.g. conventional PCR, TaqMan PCR, ISH probe).
2. To develop and validate PCR assays for the detection of the abalone virus.
3. To develop an in situ hybridisation assay specific for the abalone herpesvirus
4. To determine which abalone tissues provide accurate diagnosis of infection.
5. To develop a diagnostic antiserum for use as an immunohistochemical reagent in diagnosis of abalone herpesvirus infections.
6. 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 tests 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.

3. A further output from this project will be a newly qualified diagnostic virologist trained in modern diagnostic techniques that would be suitable for a diagnostic/research position in a State laboratory or research institution.

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 DNA 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 Software version 1.5 (PE Applied Biosystems), primers and probes will be designed to detect the abalone virus.
-Herpesvirus nucleic acid sequences suitable for use as a diagnostic probe will be developed for in situ hybridisation.
-Infection trials of healthy abalone will be performed within the AAHL biosecure facility to determine which abalone tissues are the most appropriate for sampling during an active surveillance program of, for example, wild broodstock.
-Either virus will be purified from host tissue or recombinant virus proteins will be engineered to provide antigens for production of diagnostic polyclonal antiserum in rabbits.
-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
E-MAIL: joanne.slater@csiro.au

No later than cob on Friday 23 June 2006.

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DELETION

From: Crane, Mark (LI, Geelong)
Sent: Thursday, 25 May 2006 1:49 PM
To: [s22]
Cc: Corbeil, Serge (LI, Geelong); 'malcolm.lancaster@dpi.vic.gov.au'; 'annflem@bigpond.net.au'
Subject: FW: herpes viruses
Attachments: Arzul et al Virol 290 342 2001.pdf; Abalone herpes-like virus DAO 65 23-27 2005.pdf; Arzul et al JGV 82 865 2001.pdf

Follow Up Flag: Follow up
Flag Status: Completed

DELETION

Thanks, [s22] We do have these references - in fact, it is these that we are basing our molecular work on - as, I presume, Gribbles is too.

Cheers

Mark

MARK CRANE Ph.D.
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DELETION

s22

Virology 290, 342–349 (2001)
doi:10.1006/viro.2001.1186, available online at http://www.idealibrary.com on IDEAL®

French Scallops: A New Host for Ostreid Herpesvirus-1

Isabelle Arzul,* Jean-Louis Nicolas,† Andrew J. Davison,‡ and Tristan Renault*,[1]

*IFREMER, Laboratoire de Génétique et Pathologie, 17390 La Tremblade, France; †IFREMER, Laboratoire de Physiologie des Invertébrés, 29280 Plouzané, France; and ‡MRC Virology Unit, Church Street, Glasgow G11 5JR, United Kingdom

Received August 13, 2001; returned to author for revision September 4, 2001; accepted September 12, 2001

Sporadic high mortalities were reported among larval French scallops (*Pecten maximus*). Electron microscopy of moribund larvae revealed particles with the characteristics of a herpesvirus in association with cellular lesions. PCR and DNA sequencing showed that the virus is a variant of ostreid herpesvirus-1 that has already been described in clams and oysters. This is the first description of a herpesvirus infection of a scallop species. The virus was transmitted successfully from an extract of infected scallop larvae to uninfected scallop or oyster (*Crassostrea gigas*) larvae, demonstrating that it is able to infect both species. Detection of viral DNA in asymptomatic adult scallops by *in situ* hybridisation indicates that the herpesvirus may have been transmitted from adults to larvae. It is notable that, unlike most herpesviruses, this virus has a wide host range reflected by its ability to infect several species of marine bivalve.

Key Words: herpesvirus; scallop; *Pecten maximus*; OsHV-1; polymorphism; transmission; host range; bivalve.

INTRODUCTION

Viruses morphologically similar to members of the family *Herpesviridae* have been identified in various marine bivalve species around the world (Renault, 1998). The first observation was reported in *Crassostrea virginica* adults (Farley *et al.*, 1972). Subsequently, herpes-like viruses were associated with high mortality rates in other cultivated oyster species including *Ostrea edulis* (Comps and Cochennec, 1993; Renault *et al.*, 2000a) and *C. gigas* in French (Nicolas *et al.*, 1992; Renault *et al.*, 1994a) and New Zealand (Hine *et al.*, 1992) hatcheries. Herpes-like viruses were also observed in haemocytes of *O. angasi* adults in Australia (Hine and Thorne, 1997) and in New Zealand in flat oysters, *Tiostrea chilensis* (Hine *et al.*, 1998). One of these viruses, isolated from moribund larval *C. gigas*, has been characterised as ostreid herpesvirus-1 (OsHV-1) (Minson *et al.*, 2000), and its 207-kb genome has been completely sequenced (A. J. Davison, unpublished data). Herpesviruses have also been characterised in nonostreid bivalves, the European clam, *Ruditapes decussatus*, and Manila clam, *R. philippinarum* (Renault and Arzul, 2001; Renault *et al.*, 2001). A variant of OsHV-1 (hereafter termed OsHV-1var) was detected in Manila clam and Japanese oyster larvae which presented concomitant mortalities in 1997 in a French hatchery (Arzul *et al.*, 2001b). OsHV-1 and OsHV-1var are representatives of a single viral species that may be the ubiquitous cause of observed herpesvirus infections of marine bivalves.

In September 2000, high mortality rates (100%) occurred among certain batches of scallop, *Pecten maximus*, larvae in a Breton commercial hatchery in France. Scallop aquaculture was developed locally in Brittany for restocking natural French environments, as this species had been seriously depleted in the 1980s owing to intense fishing pressure and unfavourable meteorological conditions. Breeding of larval and postlarval scallops is intensive and at risk from epizootic diseases, especially those caused by bacteria such as the *Vibrio* species (Nicolas *et al.*, 1996). With the exception of small DNA-negative virus-like particles reported in *P. noraezelandiae* (Hine and Wesney, 1997), viral aetiology has not been linked to scallop mortalities. We describe here for the first time a herpesvirus that infects *P. maximus* and show that it is ostensibly the same at OsHV-1var. Herpesviruses are usually detected in larvae and spat, but some reports concern adults (Farley *et al.*, 1972; Hine and Thorne, 1997), suggesting that bivalve herpesviruses are able to persist in adults without mortality (I. Arzul, unpublished data). Adult scallops belonging to the batch which produced infected larvae were analysed by *in situ* hybridisation and shown to be carrying the herpesvirus.

RESULTS

Analysis of moribund larvae

Detection of Herpes-like Virus Particles by TEM. Viral particles were observed in the nuclei and cytoplasm of infected cells in the connective tissues of moribund *P. maximus* larvae collected at 7 or 10 days old. Nuclear particles were circular or polygonal in shape, 74–86 nm in diameter (Fig. 1). Some nuclear particles contained an

[1] To whom correspondence and reprint requests should be addressed. Fax: (33) 5 46 36 37 51. E-mail: trenault@ifremer.fr.

0042-6822/01 $35.00

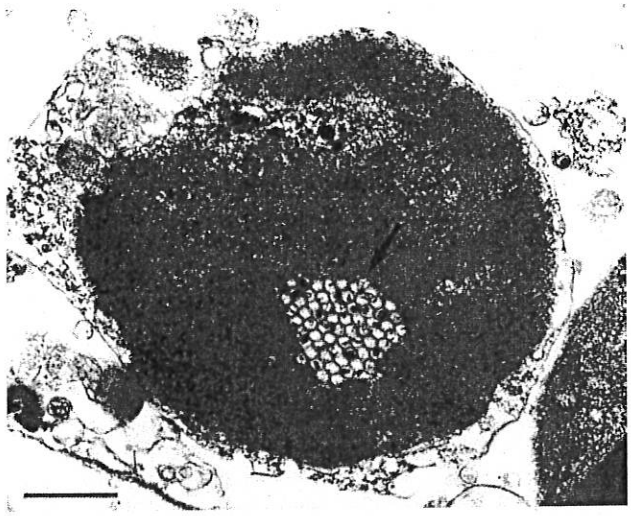

FIG. 1. Infected lysed cell showing intranuclear capsids and nucleocapsids (arrow) in condensed nucleus. Bar: 500 nm.

electron-dense structure, and other particles appeared empty (Fig. 2). Naked nucleocapsids were also observed free in the cytoplasm of lysed infected cells (Fig. 3). Enveloped virions were detected in cytoplasmic vesicles (Fig. 4) and also in perinuclear spaces (Fig. 5). The occurrence of nuclear tubular structures 45 to 55 nm in diameter was also observed (Fig. 4). Extracellular particles were usually enveloped and measured approximately 110 nm in diameter. Infected cells showed hypertrophied nuclei and marginated chromatin densely packed around the nuclear envelope (Fig. 3). Degenerating and lysing infected nuclei and cells were observed frequently (Figs. 3 and 4).

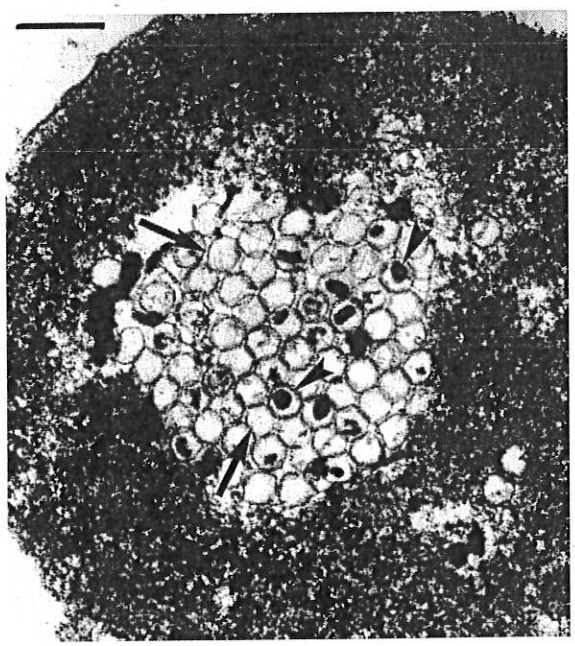

FIG. 2. Infected nucleus containing capsids (arrows) and nucleocapsids with pleomorphic cores (arrowheads). Bar: 200 nm.

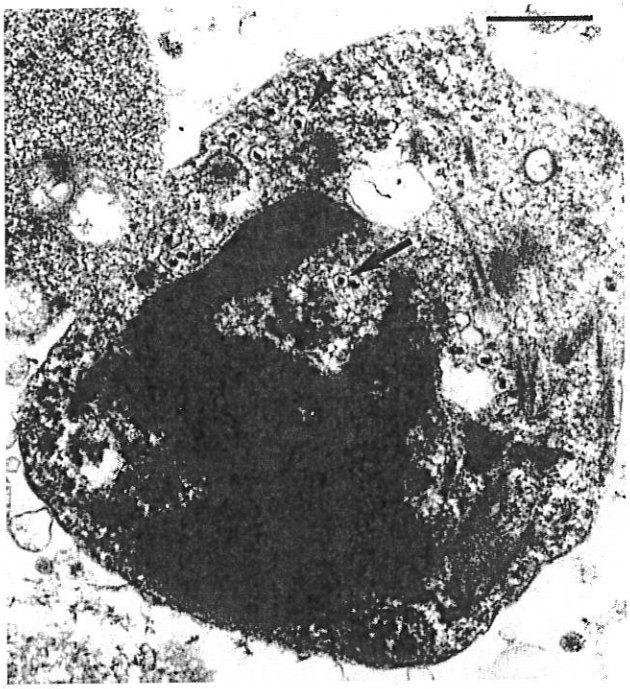

FIG. 3. Lysed cell showing intranuclear (arrow) and intracytoplasmic virions (arrowhead). Bar: 500 nm.

Detection of Oyster Herpesvirus DNA by PCR Analysis. Four samples (1–4) were analysed using primer pairs Gp3/Gp4, B3/B4, and C2/C4. Samples 1 and 2 consisted of 7-day-old moribund *P. maximus* larvae and samples 3 and 4 consisted of 10-day-old moribund larvae. The Gp3/Gp4 and B3/B4 primers amplified fragments of the ex-

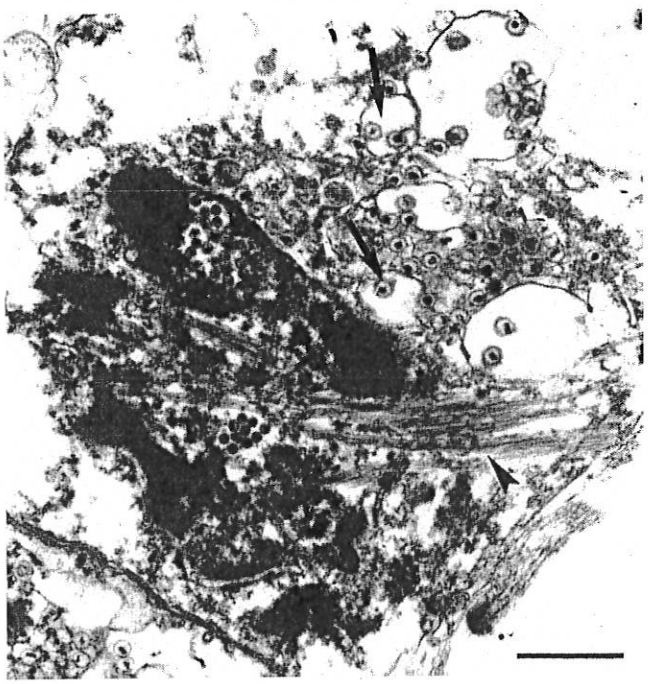

FIG. 4. Infected cell showing enveloped virions in cytoplasmic vesicles (arrows) and intranuclear tubular structures (arrowhead). Bar: 500 nm.

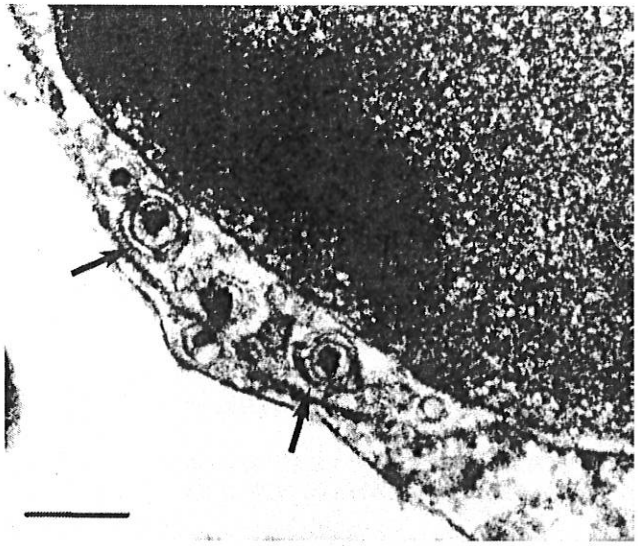

FIG. 5. Enveloped virions (arrows) showing a longitudinal section of the toroidal core within perinuclear space. Bar: 200 nm.

pected sizes from the four larval samples and OsHV-1 DNA (Figs. 6a and 6b). The C2/C4 PCR products were about 180 bp smaller than those obtained with OsHV-1 DNA (Fig. 6c). No PCR product was detected in negative controls.

Analysis of Viral DNA by Sequencing. The Gp3/Gp4 and C2/C4 PCR products derived from larval samples 3 and 4 were sequenced. Two independent plasmids were processed for each product to rule out errors induced by PCR amplification. The sequences of each pair were identical. The sequences of the Gp3/Gp4 fragments from samples 3 and 4 were identical to each other and 99% identical to the OsHV-1 sequence (Fig. 7a). The nucleotide differences resulted in seven amino acid residue substitutions, yielding 97% identity in the predicted protein sequences (Fig. 7b). The sequences of the C2/C4 fragments from samples 3 and 4 were identical to each other and to that of OsHV-1var (Arzul *et al.*, 2001b), but different from that of OsHV-1. Differences between OsHV-1 and OsHV-1var included several single nucleotide substitutions, insertions, or deletions and, more notably, a deletion of 200 bp near the C2 primer site accompanied by an insertion of 27 bp. Arzul *et al.* (2001b) registered the possibility that OsHV-1var lacks the region containing C2 and that this primer instead hybridises fortuitously to a partially matched region.

Transmission assays of viral infection

Intraspecies Transmission of Viral Infection. In the three replicates of assays 1 and 2, larvae presented high mortality rates which reached 100% at 5 days after exposure to extract from moribund *P. maximus* larvae (Table 1). Two days after exposure, the mean mortality rates were 26.7 and 22.3% for the three replicates of assay 1 and assay 2, respectively (Table 1). TEM revealed intracellular virus particles in these larvae. In the three replicates of assay 3, larvae not exposed to extract presented mean mortality rates of 2% at 2 days after exposure and 6% at 5 days after exposure (Table 1). No viral particle was observed in these larvae by TEM.

Interspecies Transmission of Viral Infection from Scallop Larvae to Oyster Larvae. Three days after exposure to extract from moribund *P. maximus* larvae (assays 1–5) or sterile seawater (assays 6–7), *C. gigas* larvae were recovered, washed three times, and prepared for PCR. The initial inoculum, the three wash samples (S1–S3), and the larvae were analysed using primer pair C2/C6. Products from the inoculum appeared 180 bp smaller than those obtained with OsHV-1 DNA (Fig. 8). Samples S1–S3 in assays 6–7 did not yield product (Table 2). For assays 1–5, the products from S1 and S2 were the same size as those from the inoculum (Table 2 and data not shown), and S3 did not yield product (Table 2). Products from larvae in assays 1–5 were the same size as those from the inoculum (Fig. 8). Larvae from assays 6–7 and all the negative controls did not yield product (Fig. 8).

Detection of oyster herpesvirus DNA in adult scallops by *in situ* hybridisation

To investigate the presence of the herpesvirus in adult *P. maximus*, 10 specimens were processed for *in situ* hybridisation using digoxigenin-labelled DNA probes. Positive reactions characterised by a purple precipitate (dark area on Fig. 9) were detected in seven animals. A similar signal was detected in infected spat, but no signal was detected when digoxigenin-labelled probe or antibody were omitted or when a digoxigenin-labelled probe specific to SVCV was used.

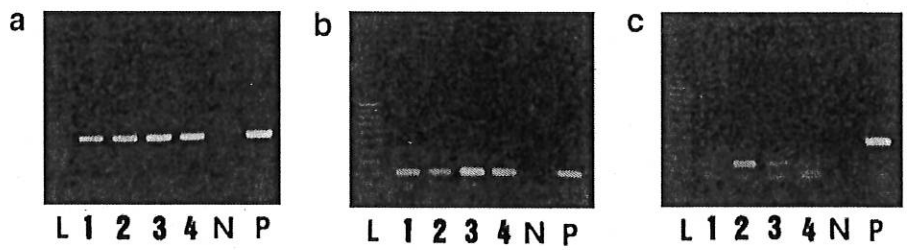

FIG. 6. PCR analysis of moribund scallop larvae using primer pairs (a) Gp3/Gp4; (b) B3/B4; and (c) C2/C4. 1–4: Samples of moribund scallop larvae. N: negative control (distilled water); P: positive control (OsHV-1 DNA); L: 100-bp ladder (Eurogentec).

a

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S    ggttgtgggtttggaaatgtAGATTTGAGCACTGTAAACACCCATGCCAGCAAAATTGGC
R    ggttgtgggtttggaaatgtAGATTTGAGCACTGTAAACACCCATGCCAGCAAAATTGGC

S    AGAAATATAAACCCTAGATTCATGGTCGGTGTTTATACAAACGATAGCAATAAGTTAATC
R    AGAAATATAAACCCTAGATTCATGGTCGGTGTTTATACAAACGATAGCAATAAGTTAATC

S    GAGGATGATATATACGGCCGTTACACAGATTCAGAATCTGCCGGTGTTATGAGAAAATGT
R    GAGGATGATATATACAGCCGTTACACAGATTCAGAATCTGCCGGTGTTATGAGAAAATGT

S    AATTTAAACGAGGTTAAAACCACTCCCCAGGAAGATTGCATTCAGCCCTTTTGCACCAAA
R    AATTTAAACGAGGTTAAAACCACTCCCCAGGAAGATTGCATTCAGCCCTTTTGCACCAAA

S    GGAACAGTGTATGGAAATAACCTCGTTTATGGAAGTAGATTACGACGTTTCAGTAGGAGC
R    GGAACAGTGTATGGAAATAACCTCGTTTATGGAAGTAGATTACGATGTTTCAGTAGGACC

S    AGGTGTTCACAGAGAAGTAGAACCGTTCCTCAATCAGTCCCTTGGTATATACCATCTGGA
R    AGGTGTTCACAGAGAAGTAGAACCGTTCCTCAATCAGTCCCTTGGTATATACCATCTGGA

S    TTTACAGGAAACCGGTTTATGTACCTGGATAATAGACTGGGATATCTTTTGGGATTAGAC
R    TTTACAGGAAAACAGTTTATGTACCTGGATAATAGACTGGGATATCTTTTGGGATTAGAC

S    CTTACCACGGCTATTTTTAAATATACCCCAATTGTTGTCGGACATATAGTAAGTGAATAC
R    CTTACCACGGCTATTTTTAAATATACCCCAATTGTTGTCGGACATATAGTAAGTGAATAC

S    CTGACGGGAATCATGAACTATAAGCGTCTCAGTGTCAGGAAAGGACCGAATATAGACATG
R    CTGACGGGAATCATGAACTATAAGCGTCTCAGTGTCAGGAAAGGACCGAATATAGACATG

S    CGAGGTATTATAGGTGGAGAAATCAAAATGATATTGATAAGAAACTACAGAAAGATGTTG
R    CGAGGTATTATAGGTGGAGAAATCAAAATGATATTGATAAGAAACTACAGAAAGATGTTG

S    GACATGAGTGGATTTACACTTTTGCCGGCGAATGGATGTTATGTCACAGTGATAAAATTC
R    GACATGAGTGGATTTACACCTTTGCCGGTGAATGGATGTTATGTCACAGTGATAAAATTC

S    ATCGGGGATAAACGCGTTtttaatcgagtttggacgcc
R    ATCGGGGATAAACGCGTTtttaatcgagtttggacgcc
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b

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S    GCGFGNVDLSTVNTHASKIGRNINPRFMVGVYTNDSNKLIEDDIYGRYTDSESAGVMRKC
R    GCGFGNVDLSTVNTHASKIGRNINPRFMVGVYTNDSNKLIEDDIYSRYTDSESAGVMRKC

S    NLNEVKTTPQEDCIQPFCTKGTVYGNNLVYGSRLRRFSRSRCSQRSRTVPQSVPWYIPSG
R    NLNEVKTTPQEDCIQPFCTKGTVYGNNLVYGSRLRCFSRTRCSQRSRTVPQSVPWYIPSG

S    FTGNRFMYLDNRLGYLLGLDLTTAIFKYTPIVVGHIVSEYLTGIMNYKRLSVRKGPNIDM
R    FTGKQFMYLDNRLGYLLGLDLTTAIFKYTPIVVGHIVSEYLTGIMNYKRLSVRKGPNIDM

S    RGIIGGEIKMILIRNYRKMLDMSGFTLLPANGCYVTVIKFIGDKRVFNRVWT
R    RGIIGGEIKMILIRNYRKMLDMSGFTPLPVNGCYVTVIKFIGDKRVFNRVWT
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FIG. 7. Comparison of (a) nucleotide and (b) protein sequence of the Gp3–Gp4 fragment from scallop larvae (S) and reference OsHV-1 DNA (R). Nucleotide or amino acid substitutions are underlined in the S sequence. Primer sequences are in lower case.

The abundance and intensity of the hybridisation signal in positive adult scallops varied between individuals and tissues. Labelling consisted of a small number of cells in the connective tissues of gills, gonad (Fig. 9), mantle, and muscle. Staining was detected in both the cytoplasm and the nuclei, and some stained cells exhibited anomalous chromatin patterns, including margination. In some cases, signal was also detected in epithelial cells of the gills.

DISCUSSION

Several descriptions of herpes-like virus infections in marine bivalves have been reported since 1972 in various species of oyster (Farley *et al.,* 1972; Hine *et al.,* 1992; Nicolas *et al.,* 1992; Comps and Cochennec, 1993; Renault *et al.,* 1994a; Hine and Thorne, 1997; Hine *et al.*, 1998; Renault *et al.,* 2000a) and clam (Renault and Arzul, 2001; Renault *et al.,* 2001). We now describe for the first

TABLE 1

Observed Mortalities and Transmission Electron Microscopy (TEM) Examination of *Pecten maximus* Larvae in the Context of Intraspecies Transmission Assays

Assay	Replicate	Mortality (%)[a] 2 days after exposure	Mortality (%)[a] 5 days after exposure	TEM[b]
1	A	42	100	+
	B	5	100	
	C	33	100	
2	A	5	100	+
	B	62	100	
	C	0	100	
3	A	3	6	−
	B	2	6	
	C	1	6	

Note. Assay 1: exposition of healthy larvae to a 0.22 μm filtered suspect larval extract. Assay 2: exposition of healthy larvae to a not filtered suspect larval extract. Assay 3: negative control assay, exposition of healthy larvae to sterile sea water.

[a] Cumulative mortality of scallop larvae after exposure to moribund larval scallop extract or sterile seawater.

[b] +, intracellular herpesvirus particles detected; −, herpesvirus particles not detected.

TABLE 2

PCR Analysis Using the Primer Pair C2/C6 of the Samples Collected in the Context of Interspecies Transmission Assays

	PCR analysis[a]			
Assay	S1: First wash	S2: Second wash	S3: Third wash	Larvae
1	+	+	−	+
2	+	+	−	+
3	+	+	−	+
4	+	+	−	+
5	+	+	−	+
6	−	−	−	−
7	−	−	−	−

Note. S1, S2, and S3 correspond to samples collected at each *Crassostrea gigas* larvae wash. Larvae correspond to the pellet obtained at the end of the three washes.

[a] +, PCR products similar in size to those from the inoculum. −, PCR products not obtained.

time a herpesvirus infecting a species of scallop, *P. maximus,* in association with high larval mortality rates.

The virus particles observed by TEM in moribund *P. maximus* larvae collected from a commercial hatchery resemble those of herpesviruses of higher and lower vertebrates in morphology, virogenesis, and size range (Roizman, 1982, 1990; Roizman and Baines, 1991). These general characteristics of virus particles are similar to those documented previously in other bivalve species (Renault *et al.,* 1994b, 2001; Hine and Thorne, 1997; Hine *et al.,* 1998). A variety of morphological forms corresponding to replicating virus was noted in intranuclear and intracytoplasmic locations. Two classes of nucleocapsid were observed. One had an electron-dense core and corresponds to DNA-containing capsids, and the other lacked the core. Enveloped capsids (virions) were observed in perinuclear spaces, cytoplasmic vesicles, and extracellular locations. Virus replication was associated with various cellular lesions, including cell lysis. Thus, the herpesvirus exhibits all the microscopic features of productive replication in scallop larvae. This is in contrast to another virus (infectious pancreatic necrosis virus), which has been described as being sequestered in the digestive tissues of *P. maximus* without replicating and being progressively transmitted through food chains to brown trout (Mortensen, 1993; Mortensen *et al.,* 1998).

The high mortality rate (100%) associated with detection of virus particles 5 days after exposure of healthy *P. maximus* larvae to infected larval extract fulfills Koch's postulates. Low mortality rates (6%) were observed 5 days after exposure of healthy larvae to sterile water, and no virus particles were detected. These results demonstrate that the herpesvirus induces larval mortalities and

FIG. 8. PCR analysis of samples subjected to interspecies transmission assays using primer pair C2/C6. I: inoculum of moribund scallop larval extract; 1–5: *C. gigas* larvae exposed to the inoculum; 6–7: *C. gigas* larvae exposed to sterile water; A–C: negative controls (distilled water); P: positive control (OsHV-1 DNA); L: 100-bp ladder (Eurogentec). The upper inverted comma indicates the positive control. The lower inverted comma indicates PCR products from samples.

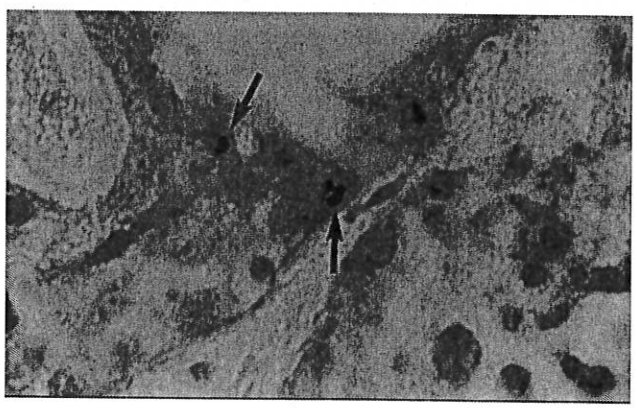

FIG. 9. Positive cells characterised by a nuclear dark staining in gonad connective tissues from adult scallop (arrows).

can be transmitted horizontally from infected to healthy scallop larvae. Variable mortality rates (0–62%) in larvae at 2 days after infection were probably due to variation in the quantity of infectious virus particles present in the inocula.

Infected larvae were analysed by PCR and DNA sequencing to assess whether the scallop herpesvirus is similar to OsHV-1. The ability to generate Gp3/Gp4 and B3/B4 PCR products of the expected sizes using primers based on the OsHV-1 sequence indicates that the scallop virus is very similar to OsHV-1. However, the sequence of the Gp3/Gp4 product shows that the scallop virus is closely related, but not identical, to OsHV-1. Unusually, each of the seven nucleotide substitutions is predicted to result in an amino acid substitution. The C2/C4 product was the same size as that for OsHV-1var (Arzul *et al.*, 2001b), 180 bp smaller than that for OsHV-1, and identical in sequence to that of OsHV-1var. Thus, it appears that OsHV-1var, which has been shown previously to infect oyster and clam larvae, also infects scallop larvae. It is not known whether OsHV-1 can infect scallop larvae.

The original source of OsHV-1var in the Breton hatchery, in which *P. maximus* is the only species bred, is unknown. However, the observations that this virus can infect several species in the field and that the scallop virus could be transmitted to *C. gigas* larvae under laboratory conditions are entirely consistent with acquisition from another bivalve species. A possible role for vertical transmission from adults to larvae is supported by the detection of viral sequences in asymptomatic scallops from the same batch of adults that produced the larvae. It is notable that the connective tissue of the gonad was among the regions that contained infected cells. Vertical transmission has also been implicated for OsHV-1 in *C. gigas* (Le Deuff *et al.*, 1996), and viral proteins and DNA have been detected in healthy adult specimens of this species (I. Arzul, unpublished data).

This report extends the number of marine bivalve species that can be infected by OsHV-1var and adds to the pathogens already faced by *P. maximus* in commercial aquaculture. It highlights the risks of breeding different species in the same hatchery and indicates the importance of exercising strict precautionary measures to prevent the spread of herpesvirus infections between monocultures. It also stimulates further speculation that OsHV-1 and OsHV-1var are derived from a parental, and perhaps benign, bivalve herpesvirus that has been selected for virulence and wide host range by the practices of modern shellfish farming (Arzul *et al.*, 2001b).

MATERIALS AND METHODS

Animals

Moribund *P. maximus* larvae, 7 or 10 days old, were collected in September 2000 from a hatchery located in Brittany (France). Before sampling, the larvae presented high mortality rates, which reached 100% in all batches by 10 days after fertilisation.

Ten adults from the same batch that produced the larvae were collected for *in situ* hybridisation analysis. These animals did not present any mortality or other symptoms of disease.

Intraspecies transmission

Seven-day-old *P. maximus* larvae from a Breton hatchery were placed at a concentration of five larvae per milliliter in 2-l flasks containing 0.22-μm filtered seawater. They were incubated at 18°C and fed with a mixture of three algae (*Pavlova lutheri*, *Isochrysis affinis galbana*, and *Skeletonema costatum* or *Chaetoceros calcitrans*) at a concentration of 20 cells/μl for each species. Algae and antibiotic (see below) were added three times per week, and the culture water was completely renewed three times per week.

Three different assays, each replicated three times, were carried out using 7-day-old healthy larvae. In assays 1 and 2, larvae were exposed to 1 ml of extract obtained by grinding 10^5 moribund *P. maximus* larvae in 20 ml of sterile seawater using a Dounce homogeniser. In assay 1, the ground larvae were filtered through a 0.22-μm filter to remove bacterial contaminants. In assay 2, the ground larvae were not filtered. In assay 3 (the negative control), healthy larvae were maintained without adding ground larval extract. Chloramphenicol (4 mg/l) was included in all assays to reduce mortalities related to bacterial infections (Nicolas *et al.*, 1996).

Interspecies transmission

Moribund *P. maximus* larvae (400 mg) from a Breton hatchery were ground in 20 ml of seawater using a Dounce homogeniser and filtered through a 0.22-μm filter to remove bacterial contaminants. One microliter of inoculum was sampled for PCR. Five 1-l flasks containing 3-day-old axenic *C. gigas* larvae were inoculated with 8 ml of ground larval extract (assays 1–5), and two 1-l flasks containing 3-day-old axenic *C. gigas* larvae were inoculated with 8 ml of sterile seawater (assays 6–7). Three days after exposure, the larvae were recovered, concentrated, and washed three times in filtered seawater by centrifugation (200 g for 15 min) as described by Arzul *et al.* (2001a). One microliter of seawater was collected for PCR analysis after each wash. After washing, larvae were treated for PCR analysis (Renault *et al.*, 2000b).

Transmission electron microscopy

Moribund larvae were fixed directly in 3% glutaraldehyde in 0.2 M sodium cacodylate (pH 7.2). Larval samples were then processed as described previously (Renault *et al.*, 1994a). Ultrathin sections were stained

with uranyl acetate and lead citrate and examined by transmission electron microscopy (TEM) using a JEOL JEM 1200EX instrument operating at 80 kV.

PCR and sequence analysis

Larval samples were prepared by grinding frozen larvae in double-distilled water, boiling, and centrifuging (Renault *et al.*, 2000b). Supernatants were immediately diluted 10-fold in double-distilled water and frozen at −20°C. Oligonucleotide primers were designed on the basis of the OsHV-1 genome sequence. Four primer pairs were used, as follows: Gp3 (5′-GGT TGT GGG TTT GGA AAT GT-3′) and Gp4 (5′-GGC GTC CAA ACT CGA TTA AA-3′) (698-bp product), B3 (5′-GTG GAG GTG GCT GTT GAA AT-3′) and B4 (5′-ACT GGG ATC CGA CTG ACA AC-3′) (207-bp product), C2 (5′-CTC TTT ACC ATG AAG ATA CCC ACC-3′) and C4 (5′-GCA GTT GTG GTA TAC TCG AGA TTG-3′) (352-bp product), and C2 and C6 (5′-GTG CAC GGC TTA CCA TTT TT-3′) (710-bp product; overlaps the C2/C4 product). The Gp locus (at approximately 134,000 bp in the genome) encodes part of a putative glycoprotein; the B locus (146,900 bp) encodes part of a putative inhibitor of apoptosis protein, and the C locus (present twice in the genome at 4500 and 178,500 bp) encodes parts of two proteins of unknown functions (Arzul *et al.*, 2001b). PCR reactions were carried out and the products analysed on 1% agarose gels as described previously (Renault *et al.*, 2000b). The positive control consisted of 10 ng/μl OsHV-1 DNA extracted from purified virus particles (Le Deuff and Renault, 1999).

Gp3/Gp4 and C2/C4 PCR products derived from two moribund larval scallop samples were ligated into linearised pT-Adv vector (Clontech). Ligated DNA was transformed into competent *Escherichia coli* TOP10F′. Relevant portions of selected plasmids were sequenced using an ABI PRISM sequencing kit (Perkin–Elmer).

In situ hybridisation

In situ hybridisation was performed according to a previously described protocol (Renault and Lipart, 1998), except that 896-bp probes were produced by PCR using OsHV-1 DNA as template (0.1 ng/reaction), primers C1 (5′-TTC CCC TCG AGG TAG CTT TT-3′) and C6 and digoxigenin-11-dUTP (Boehringer Mannheim) in a buffer containing 1.5 mM $MgCl_2$. Detection was performed using an alkaline phosphatase conjugated mouse IgG antibody against digoxigenin (1:500) incubated in NBT/BCIP (1:50). The slides were stained with Bismarck brown yellow and mounted in Eukitt resin. Negative controls included samples processed without including digoxigenin-labelled probes or without antibody. An additional negative control consisted of samples hybridised with a digoxigenin-labelled probe specific to Spring Viraemia of Carp Virus (SVCV) kindly supplied by Dr. R. M. Le Deuff (CEFAS, Weymouth, U.K). A positive control consisted of *C. gigas* juveniles shown to be infected with OsHV-1 by TEM and PCR.

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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.

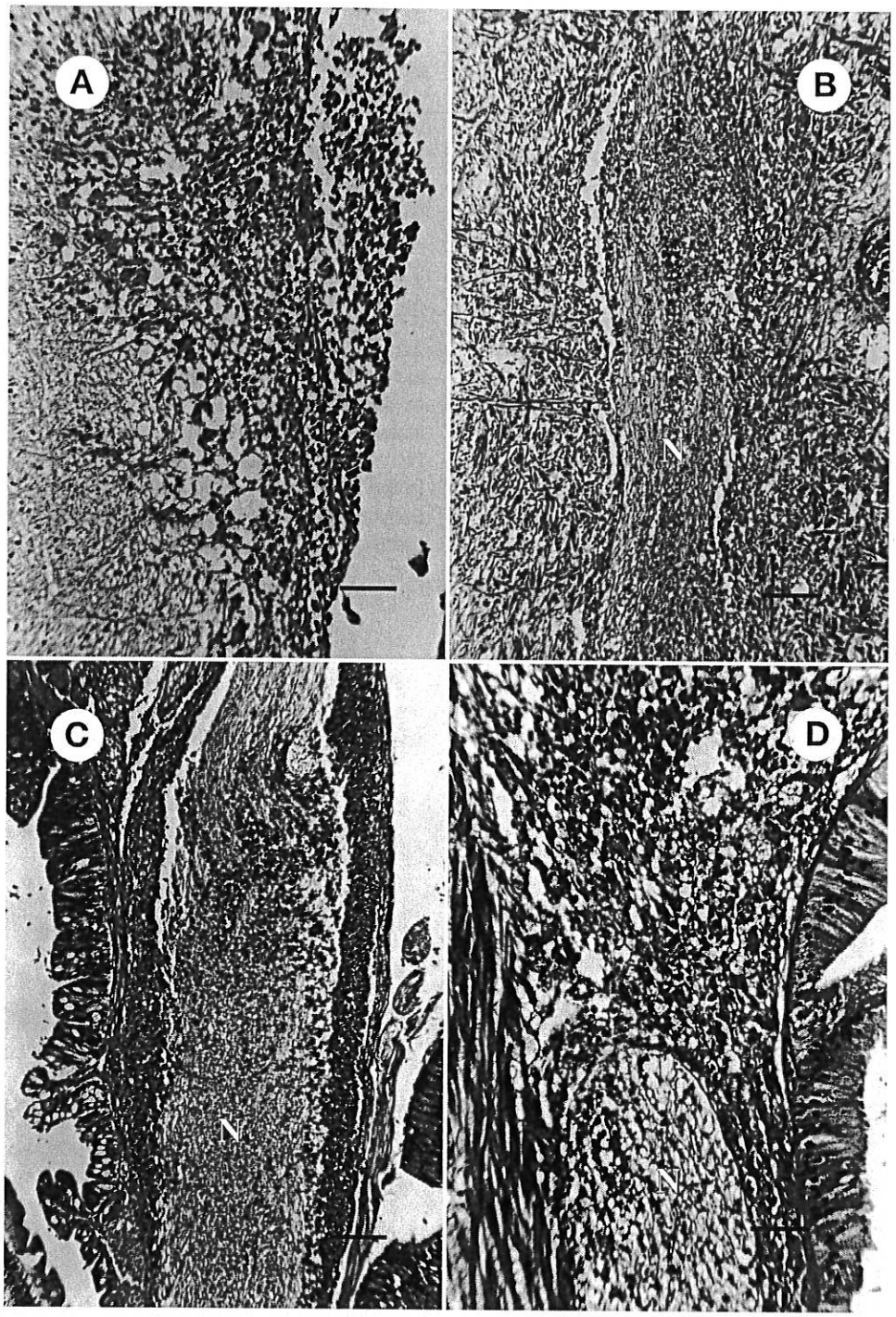

Fig. 1. *Haliotis diversicolor supertexta*. (A) Histological examination of the cerebral ganglion revealing lesions in the cerebral ganglion and surrounding neurilemma. Diffuse necrosis accompanied with infiltration of haemocytes was prominent in the tissue. Degenerated neurosecretory cells (arrow) were 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

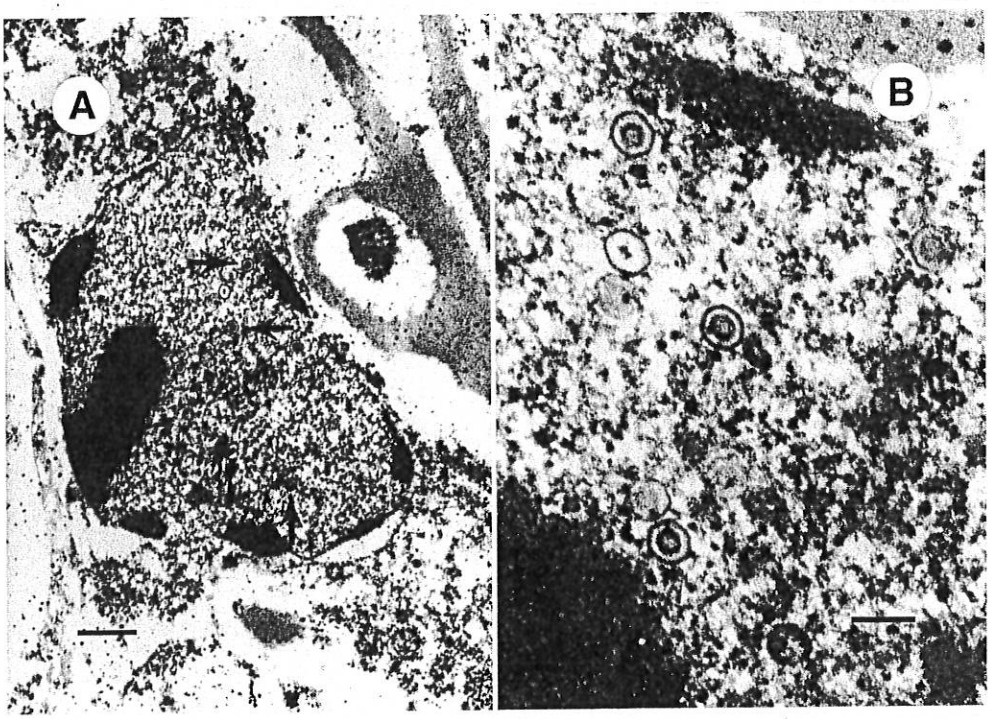

Fig. 2. *Haliotis diversicolor supertexta*. (A) TEM examination of cerebral ganglions showing viral particles (arrows) within degenerated cells. Scale bar = 416 µm. (B) The viral particles were hexagonal, 90 to 100 nm in diameter and had a single coat. Scale bar = 125 µm

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.

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Evidence for interspecies transmission of oyster herpesvirus in marine bivalves

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Since 1991, numerous herpesvirus infections associated with high mortality have been reported around the world in various marine bivalve species. In order to determine whether these infections are due to ostreid herpesvirus-1 (OsHV1), a previously characterized pathogen of the Japanese oyster (*Crassostrea gigas*), PCR analysis was carried out on 30 samples of larvae collected from four bivalve species (*C. gigas*, *Ostrea edulis*, *Ruditapes decussatus* and *Ruditapes philippinarum*), most exhibiting mortality prior to collection. All samples were shown to be infected by OsHV1. Viral genomes in three samples of *C. gigas* and three of *R. philippinarum* that originated from the same hatchery were unusual in bearing a deletion of at least 2·8 kbp in an inverted repeat region. The results demonstrate that OsHV1 is capable of infecting several bivalve species, and this raises the possibility that interspecies transmission may be promoted by intensive rearing in modern hatcheries.

The family *Herpesviridae* comprises over 120 viruses that infect a wide range of vertebrates (Minson *et al.*, 2000). Particles with herpesvirus morphology have also been observed in invertebrates, specifically in marine bivalve species. The first such observation was made with adults of the Eastern oyster, *Crassostrea virginica* (Farley *et al.*, 1972). Subsequently, herpesviruses were detected in other farmed oyster species, such as the Japanese oyster, *Crassostrea gigas* (Hine *et al.*, 1992; Nicolas *et al.*, 1992; Renault *et al.*, 1994*a*, *b*), and the European flat oyster, *Ostrea edulis* (Comps & Cochennec, 1993; Renault *et al.*, 2000*b*). These infections were associated with high levels of mortality in larvae and juveniles. More recently, herpesvirus infections have also been described in *Ostrea angasi* adults (Hine & Thorne, 1997), in *Tiostrea chilensis* larvae (Hine *et al.*, 1998) and in the larvae of two clam species, the grooved carpet shell, *Ruditapes decussatus* (T. Renault, unpublished data), and the Manila clam, *Ruditapes philippinarum* (Renault, 1998).

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Sporadic virus infections of *C. gigas* larvae and juveniles have been observed in France each summer since 1991 (Renault, 1998). In the case of larvae, symptoms typically appear 4–5 days after fertilization. Larvae reduce their feeding and swimming activities and sediment from the water (Le Deuff *et al.*, 1994). Substantial mortality occurs by day 6, reaching 100% by days 8–12 (Renault *et al.*, 1994*a*, 1995). A causative role for the virus in larval mortality is supported by transmission experiments (Le Deuff *et al.*, 1994). In the case of juveniles, high mortality levels (80–90%) occur sporadically among farmed spat at months 3–12 (Renault *et al.*, 1994*b*). Prior to death, no gross physiological signs are detectable. The main histological changes consist of enlarged and abnormally shaped nuclei and abnormal chromatin patterns throughout the connective tissues (Renault *et al.*, 1994*b*, 1995). Mortality or morbidity has not been reported in adult oysters in France.

As histological lesions are not specific to herpesvirus infection and no bivalve cell line is available to facilitate virus culture, infections are routinely diagnosed by detection of virus particles by transmission electron microscopy (TEM). The size, structure and sequence of the genome support the hypothesis that the virus that infects *C. gigas* larvae is a member of the *Herpesviridae* (Le Deuff & Renault, 1999; A. J. Davison, unpublished data), now termed oyster herpesvirus or ostreid herpesvirus-1 (OsHV1; Minson *et al.*, 2000). Specific PCR tools have been developed to enable rapid diagnosis of OsHV1 in large numbers of samples (Renault & Lipart, 1998; Renault *et al.*, 2000*a*).

Herpesviruses have been detected in several bivalve species in different parts of the world, but it is not known whether these agents represent different viruses, as might be anticipated from the fact that vertebrate herpesviruses are invariably associated closely with individual host species. In order to answer this question, samples of larvae from different bivalve species obtained from different locations were analysed by PCR, restriction endonuclease digestion of PCR products and DNA sequencing.

Animals were obtained at 1–26 days after fertilization from three hatcheries on the Atlantic and Channel coasts of France; in Normandy, Vendée (370 km south of Normandy) and

Charente-Maritime (530 km south of Normandy). Samples were collected during the period 1995–1999, in some cases from broods that presented abnormally high mortality, and were stored at −20 °C. Thirty infected samples were selected for analysis (Table 1). Twenty consisted of *C. gigas* larvae that had sedimented before sampling. Three of these samples (10–12) and the three samples of *R. philippinarum* larvae (13–15) originated from the same hatchery and were obtained during an episode of high mortality. Four samples consisted of *O. edulis* larvae that did not exhibit mortality (1–3 and 29). Lastly, three samples (4–6) consisted of *R. decussatus* larvae presenting high mortality.

Samples of larvae were prepared as described previously (Renault *et al.*, 2000*a*). Briefly, 50 mg samples of frozen larvae were macerated by grinding in 50 µl double-distilled water with a disposable tissue homogenizer, boiled and centrifuged. Supernatants were diluted tenfold in double-distilled water and stored at −20 °C. PCR primers were designed for three regions of the genome, based on initial OsHV1 genome sequence data. The sequences of the primers are shown in Fig. 1(*c*). After the analysis was carried out, determination of the DNA sequence of OsHV1 was completed (A. J. Davison, unpublished data), enabling retrospective location of the three regions on the genome (Fig. 1*a*, *b*). Region A encodes an inhibitor of apoptosis belonging to the IAP family, members of which include proteins specified by cellular genomes and by other viruses of invertebrates. Region B encodes a protein of unknown function. Region C encodes two proteins of unknown function and, being located in an inverted repeat, is present as two copies in the genome.

Primary (not nested) PCR was employed for all analyses. Thermal cycling (35 cycles) was carried out as described previously (Renault *et al.*, 2000*a*) and products were visualized by agarose gel electrophoresis alongside molecular mass markers. Reference OsHV1 DNA purified from infected *C. gigas* larvae (Le Deuff & Renault, 1999), which was also used for genome sequencing, constituted the positive control and the negative control was double-distilled water. Negative controls were included at every fifth sample during each PCR experiment. It should be noted that specimens were selected on the basis that they were likely to be positive, and that negative samples are detected frequently in routine PCR diagnosis carried out in the laboratory. In some experiments, PCR products were analysed by restriction endonuclease digestion and Southern blot hybridization or were cloned and sequenced.

The primers A3/A4 and B1/B2 amplified fragments of the sizes predicted for OsHV1 from all larval samples and from reference DNA (summarized in Table 1). In contrast, the primers C1/C6 amplified the predicted fragment from only 24 samples. The six negative samples originated from two bivalve species obtained from the same hatchery (10–12 and 13–15) and were shown to contain herpesvirus particles by TEM (Table 1). Further investigations were undertaken on these samples, with the primer pairs C2/C4, C2/C6 and C1/C4 (Fig. 2*a*). No product was obtained with samples amplified with C1/C4. Products were obtained with C2/C6 and C2/C4, but were aberrant in being about 180 bp smaller than those obtained with reference DNA, at 530 and 170 bp, respectively. The negative controls did not yield PCR products in any experiment, indicating that laboratory contamination had not occurred.

PCR products of the anticipated sizes from the A, B and C regions were subjected to restriction endonuclease digestion in order to assess their origins. Digestion profiles of the A3/A4 and B1/B2 products with two endonucleases, each cleaving at between one and three sites, were identical to each other and were exactly as predicted from the genome sequence (data not shown). Digestion of C2/C6 products with three restriction endonucleases (*Xho*I, *Sty*I and *Acs*I) yielded the expected restriction patterns for 24 samples (data not shown). Southern blot hybridization analysis of the smaller PCR products obtained with the primer pair C2/C6 from the six aberrant samples (samples 10–15) yielded fragments consistent with the loss of approximately 180 bp at or near the end of the fragment defined by C2 (Fig. 2*b*). Digestion of the reference PCR product of 709 bp with *Xho*I gave fragments of 335 and 374 bp, *Sty*I yielded fragments of 258 and 451 bp and *Acs*I produced fragments of 153, 263 and 293 bp, as predicted from the genome sequence. *Xho*I generated a fragment of about 160 bp instead of 335 bp from the two aberrant samples shown (samples 11 and 14) and *Sty*I and *Acs*I digests lacked the 258 and 263 bp fragments, respectively.

C2/C4 and C2/C6 products from one of the aberrant *C. gigas* samples (sample 11), one of the aberrant *R. philippinarum* samples (sample 14) and reference DNA were cloned into plasmids and sequenced. Three independent plasmids were sequenced for each product in order to rule out errors induced by PCR amplification. As expected, the reference sequence was identical to the appropriate part of the genome sequence. The sequence of the aberrant fragment from *C. gigas* was identical to that from *R. philippinarum*, but differed from that of the reference (Fig. 1*d*). The differences included several single nucleotide substitutions, insertions and deletions and, more notably, a deletion of 200 bp near the C2 sequence, accompanied by an insertion of 27 bp. These differences would be expected to disrupt the functions of the two proteins encoded by the C region. The provenance of the 27 bp insert is unknown; it is not present in the entire reference virus genome. It is possible that primer C2 functioned as a result of fortuitous hybridization to a non-specific sequence located nearer to the C4 and C6 primer recognition sites in the aberrant genome than the cognate sequence in the OsHV1 genome. Further assays with combinations of six additional primers up to 2·8 kbp upstream from the deletion end-point failed to yield products from the aberrant samples, indicating that a region of the inverted repeat of at least 2·8 kbp is absent from the virus genome. PCR products of the expected sizes were obtained when reference DNA was amplified with these primers. The

Table 1. PCR and TEM analysis of larval bivalve samples

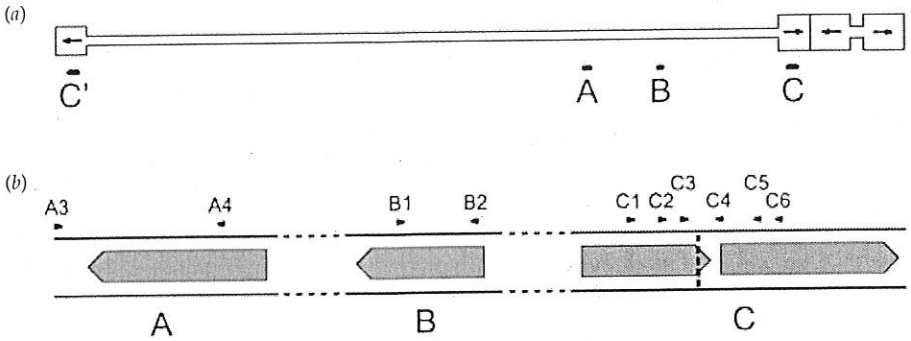
Sample	Hatchery*	Bivalve species	Date of sampling	Mortality before sampling†	TEM analysis‡	Product with PCR primers§		
						A3/A4	B1/B2	C1/C6
1	N	*O. edulis*	28/04/1997	−	+	+	+	+
2	N	*O. edulis*	28/04/1997	−	+	+	+	+
3	N	*O. edulis*	28/04/1997	−	+	+	+	+
4	N	*R. decussatus*	30/09/1998	+	ND	+	+	+
5	N	*R. decussatus*	30/09/1998	+	ND	+	+	+
6	N	*R. decussatus*	30/09/1998	+	ND	+	+	+
7	N	*C. gigas*	07/06/1997	+	+	+	+	+
8	N	*C. gigas*	07/06/1997	+	+	+	+	+
9	N	*C. gigas*	07/06/1997	+	+	+	+	+
10	N	*C. gigas*	15/06/1997	+	+	+	+	−
11	N	*C. gigas*	15/06/1997	+	+	+	+	−
12	N	*C. gigas*	15/06/1997	+	+	+	+	−
13	N	*R. philippinarum*	15/06/1997	+	+	+	+	−
14	N	*R. philippinarum*	15/06/1997	+	+	+	+	−
15	N	*R. philippinarum*	15/06/1997	+	+	+	+	−
16	V	*C. gigas*	08/07/1995	+	+	+	+	+
17	V	*C. gigas*	09/07/1995	+	+	+	+	+
18	V	*C. gigas*	10/07/1995	+	+	+	+	+
19	V	*C. gigas*	10/07/1995	+	+	+	+	+
20	V	*C. gigas*	25/07/1995	+	+	+	+	+
21	V	*C. gigas*	25/07/1995	+	+	+	+	+
22	V	*C. gigas*	27/07/1995	+	+	+	+	+
23	V	*C. gigas*	04/08/1995	+	+	+	+	+
24	V	*C. gigas*	04/08/1995	+	+	+	+	+
25	V	*C. gigas*	24/08/1995	+	+	+	+	+
26	V	*C. gigas*	24/08/1995	+	+	+	+	+
27	V	*C. gigas*	24/08/1995	+	+	+	+	+
28	V	*C. gigas*	17/03/2000	+	ND	+	+	+
29	C	*O. edulis*	11/04/2000	−	ND	+	+	+
30	C	*C. gigas*	01/04/1997	+	+	+	+	+

* Abbreviations: N, Normandy; V, Vendée; C, Charente-Maritime.
† Scored as: +, mortality and sedimentation observed; −, mortality and sedimentation not observed.
‡ Scored as: +, virus particles detected; ND, analysis not done.
§ Scored as: +, product obtained; −, product not obtained.

upstream end-point of the deletion was not determined. Sequencing was also carried out for 380 bp at each end of the 1001 bp PCR product from the A region and for 332 bp in the B region. The reference sequence was identical to the appropriate part of the genome sequence in both regions. Samples 11 and 14 were identical to each other in regions A and B, region B being identical to the reference sequence and region A differing by a single synonymous nucleotide substitution.

The results of this study indicate that four bivalve species belonging to three genera were infected with OsHV1 and that infection of more than one species was not confined to a single hatchery. Infection with the variant of OsHV1 was detected in two bivalve species in a single hatchery during one episode of mortality. The variant did not persist to later episodes, however, as infected samples taken from the same hatchery at subsequent dates yielded PCR products characteristic of reference DNA (data not shown). We conclude that OsHV1 (and the variant) may be transmitted from one species of bivalve to another, and therefore that the natural host of OsHV1 must be considered a matter of uncertainty.

In the natural setting, vertebrate herpesviruses are invariably associated with a single host species. Moreover, the implication that the majority of these viruses have evolved with their hosts over long periods of time finds strong support from molecular phylogenetic studies (McGeoch & Cook, 1994; McGeoch *et al.*, 1995). Exceptionally, transmission can occur from one species to another in the context of farms or zoos. Examples include infection of humans with the simian herpesvirus B virus (Whitley, 1996) and of cattle, sheep, dogs and cats with the porcine herpesvirus pseudorabies virus (Gustafson, 1981). Interspecies transmission may also have



(c)

A3: GCCAACCGTTGGAACCATAACAAGCG
A4: GGGAATGAGGTGAACGAAACTATAGACC

B1: ATGTAATGGGTGGTGGTGCT
B2: CAACAGCTTTGGAGGTTGGT

C1: TTCCCCTCGAGGTAGCTTTT
C2: CTCTTTACCATGAAGATACCCACC
C3: GGCAAGATGAATGGCAAGAT
C4: GCAGTTGTGGTATACTCGAGATTG
C5: CCGTGACTTCTATGGGTATGTCAG
C6: GTGCACGGCTTACCATTTTT

(d)

```
                primer C2 >>
reference       CTCTTTACCATGAAGATACCCACCAATGTGGTAAAGACGGAACAATCTTTTTCTAGGATA
variant         CTCTTTACCATGAAGATACCCACCcccactgtgatatcatcgcaaatgaat.........

reference       TGGAGCTGCGGCGCTATGGATTTAACGAGTGCCACCAAAAGTTGGGATAATGATTTTAGA
variant         ...........................................................

reference       ATAGATGTGATGTGCGGCAAGATGAATGGCAAGATACACAATGAGCTATTGCCCGACCAC
variant         ...........................................................

reference       AAACCTAACGTTGTATTCGATTACGGATTAAGAAAATGGGTTCCACAATCTAAAATTAAA
variant         ..........................................ACAATCTAAAATTAAA

                          StyI AcsI
reference       AAAACCACATGGGGGCCAAGGAATTTAAA.CCCCGGGGAAAAA.GTATAAATAGGCGCGA
variant         AAA.CCACATGGGGGCCAAGGAATTTAAAGCCCCGGGGAAAAAAGTATAAATAGGCGCGA

                                                XhoI
reference       TTTGTCAGTTTAGAATCATACCCACACACTCAATCTCGAGTATACCACAACTGCTAAATT
variant         TTTGTCAGTTTAGAATCATACCCACACACTCAATCTCGAGTATACCACAACTGCTAAATT
                                                        << primer C4
                        < trinucleotide repeat >                    AcsI
reference       AACAGCATCTACTACTACTACTACTACTACTACTGAAAAAATGCAGCCTTTCACAGAATT
variant         AACAGCATCTACTACTACTACTACTA...CTGAAAAAATGCAGCCTTTCACAGAATT

reference       TTGCACCTTGACCAAAGCCATCACATCAGCCAGCAACGACTTTTTTCATCAACCAGACGAG
variant         TTGCACCTTGACCAAAGCCATCACATCAGCCAGCACCGACTTTTTTCATCAACCAGACGAG

reference       GTTAACATGCGACATTTGTAAAGAGCTCGTCTCTTTCGATTGCGAAGATAAAGTCGTGGC
variant         GTTAACATGCGACATTTGTAAAGAGCTCGTCTCTTTCGATTGCGA.GATAAAGTCGTG.C

reference       ATCATTGGCTGCAGTCAGATCTGACATACCCATAGAAGTCACG
variant         ATCATT.GCTGCAGTCAGATCTGACATACC.ATAGA.GTCACG
```

Fig. 1. (*a*) Scale diagram of the three regions (A, B and C) aligned with the 206 kbp OsHV1 genome. Two sets of inverted repeats are depicted as rectangles orientated by arrows. Region C is also present as a second copy (C'). (*b*) Scale diagrams of regions A, B and C, with PCR primers (arrowheads) aligned with predicted protein-encoding regions (shaded arrows). The horizontal dotted lines linking the three regions are of varied length. Sizes of PCR products are: A3/A4, 1001 bp; B1/B2, 464 bp; C1/C6, 896 bp. The sequence that is deleted in aberrant samples is located to the left of the vertical dashed line in region C. (*c*) Sequences of primers. (*d*) Aligned DNA sequences of part of the C region in the reference and variant genomes. Dots indicate missing nucleotides. The locations of primers C2 and C4 are shown, with their sequences in italics. The sequence of the 27 bp insert in the variant is shown in lower case. Relevant restriction endonuclease sites are underlined. The termination codon (TAA) for one coding region and the initiation codon (ATG) for the other are marked by thick underlining. The location of a trinucleotide repeat between the coding regions is indicated.

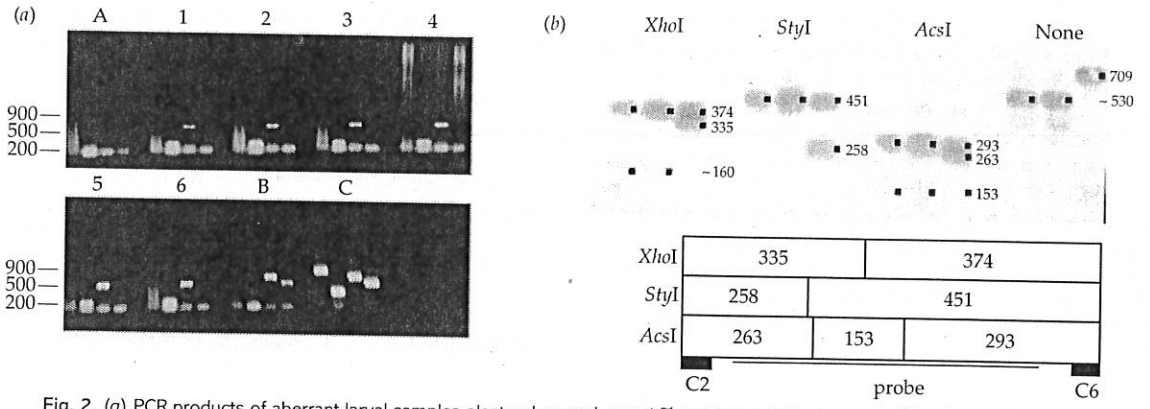


Fig. 2. (*a*) PCR products of aberrant larval samples electrophoresed on a 1% agarose gel. Each group of four lanes shows the results of amplification with C1/C6, C2/C4, C2/C6 and C1/C4, respectively. Groups: A, negative control (double-distilled water), 1–6, the samples listed in Table 1 (samples 10–12 from *C. gigas* and 13–15 from *R. philippinarum*); B, 0·5 pg reference OsHV1 DNA; C, 10 ng reference DNA. Fragment sizes are indicated in bp above the relevant bands for sample 15 and positive control C. Each lane contains a fast-migrating primer artefact. (*b*) Southern blot of restriction fragments of C2/C6 PCR products electrophoresed on a 2% agarose gel and hybridized to a digoxigenin-labelled probe made by PCR of reference DNA with primers C3/C5. Bound probe was detected by using a mouse IgG antibody against digoxigenin followed by a peroxidase-conjugated antibody against mouse IgG, and enzyme activity was visualized colorimetrically with diaminobenzidine in the presence of hydrogen peroxide. Fragments mentioned in the text and their sizes in bp are indicated by dots. Each group of three lanes shows the results for sample 11 in Table 1 (*C. gigas*), sample 14 in Table 1 (*R. philippinarum*) and reference DNA. Restriction maps of the reference PCR product deduced from the genome sequence are shown below, with fragment sizes in bp.

played a limited part in herpesvirus evolution (McGeoch *et al.*, 1995). It is possible that bivalve herpesviruses, like vertebrate herpesviruses, are confined to single host species in nature, but that intensive farming conditions, under which different bivalve species are kept in large numbers in unnaturally close proximity, promote transmission to new host species. It is also possible that OsHV1 is itself a mutant of a virus infecting a single bivalve species that has gained the ability to cross species boundaries.

We are grateful to Dr A. Gérard for facilitating this work at the IFREMER station in La Tremblade, France. We also thank F. Leroux (IFREMER, La Tremblade, France) for technical assistance. This study would not have been possible without the valuable contribution of private French shellfish farmers.

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Received 5 October 2000; Accepted 5 December 2000

s22 DELETION

-----Original Message-----
From: Mark.Crane@csiro.au [mailto:Mark.Crane@csiro.au]
Sent: Monday, 29 May 2006 10:07 AM
To: [s22]
Cc: Serge.Corbeil@csiro.au; malcolm.lancaster@dpi.vic.gov.au; Peter.Walker@csiro.au; annflem@bigpond.net.au
Subject: RE: Abalone Pathogen Pre-proposal

[s22]

Serge Corbeil and Malcolm Lancaster have put together a comprehensive preproposal for the Abalone herpsesvirus for the next FRDC funding cycle. It is a relatively big project with an appropriate budget. In my judgement, expanding it any further would compromise it getting up. Thus I recommend that we leave it as is. If there any further developments that would justify including other partners I will let you know.

Thanks for your interest in this issue.

Cheers

Mark

MARK CRANE Ph.D.
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International Fax: +61 3 52 275555
email: mark.crane@csiro.au

s22 DELETION

-----Original Message-----
From: Mark.Crane@csiro.au [mailto:Mark.Crane@csiro.au]
Sent: Wednesday, 10 May 2006 9:32 AM
To: [s22]
Cc: Serge.Corbeil@csiro.au; Clarke, Steven (PIRSA - SARDI); Peter.Walker@csiro.au
Subject: RE: Abalone Pathogen Pre-proposal

[s22]

I am really pushed for time at the moment. My concern is that Serge Corbeil with a team at AAHL is currently working on the abalone herpesvirus and, depending on progress, details of the project to be submitted for funding to FRDC for the 2007/8 cycle will change right up to the date for submission (1 November 2006). We are working with the affected farms and DPI Victoria and, depending on results, priorities are likely to change. The amount of money available from FRDC to fund aquatic animal health projects is very limited. Hopefully I will get a better idea about actual amounts later in the year. If results (or the problem gets bigger/higher priority) indicate that we can justify a bigger project with more partners then we can consider this.

I will be back on deck on 22 May and will catch up then.

Cheers

Mark

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s22

DELETION

-----Original Message-----
From: Mark.Crane@csiro.au [mailto:Mark.Crane@csiro.au]

Sent: Monday, 8 May 2006 10:58 AM
To: [DELETION] annflem@bigpond.net.au
Cc: [s22] Serge.Corbeil@csiro.au; Peter.Walker@csiro.au; Crispian@frdc.com.au
Subject: RE: Abalone Pathogen Pre-proposal

Steven,

Thank you for your email. For your information, Serge Corbeil has had a proposal to develop molecular diagnotics for Perkinsus in abalone rejected twice by FRDC over the last two years but is in fact undertaking this work with CSIRO funds. We believe it is a priority and have an on-going project approved by CSIRO. Nevertheless increased funding would speed up progress. In addition, we at AAHL are also currently undertaking similar work with the herpes-like virus in collaboration with DPI Vic and expect to submit a preproposal to the AAH Subprogram and the relevant FRABs for on-going funding of this work.

Furthermore, a current project (PI Jeremy Carson, DPIW, Tasmania) concerned with the diagnosis of Vibrios is in its final stages and the Final Report is close to being submitted.

It is unlikely that FRDC will fund more than one project in this area and so it would be appropriate to discuss further to determine how best to structure the project(s) to obtain the desired outcomes.

For your information, I will be away in UK from 11 May until 22 May.

Thank you for keeping us in the loop. Please contact Serge Corbeil and/or Peter Walker if you need to discuss this prior to my return.

Regards

Mark

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s22

DELETION

s22

DELETION

50

From: Crane, Mark (LI, Geelong)
Sent: Friday, 2 June 2006 2:34 PM
To: Corbeil, Serge (LI, Geelong)
Subject: FRDC Abalone herpesvirus preproposal

Follow Up Flag: Follow up
Flag Status: Completed

Serge,

I just rec'd a call from Malcolm and he gave me some figures for the preproposal so that the budget section could be completed. I have done this now (see attachment). You now need to officially send this to:

FRDC Aquatic Animal Health Subprogram (me and cc Joanne), as an attachment to an email (cc Malcolm Lancaster) stating that you wish it to be considered for the next FRDC funding cycle

I will then send it to the Abalone subprogram with a note that any industry funding would be used to decrease the FRDC budget component which is highly recommended to demonstrate the high priority of the project.

Mark

FRDC Abalone
herpesvirus 06060..

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AQUATIC ANIMAL HEALTH SUBPROGRAM

2007-08 Preliminary Research Proposal

Project Title

Aquatic Animal Health Subprogram: Development and application of molecular diagnostic procedures for 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: 30-06-2009

Preliminary Budget

FRDC Contribution	2006-07	2007-08	2008-09	2009-10	TOTAL $$s
Salaries and on costs (AAHL)	39 149	81 429	44 915		165 493
Salaries and on costs (DPI Vic – PhD student)		28 000	29 000	30 000	87 000
Travel		2 000	2 000	2000	6 000
Operating	12 858	38 644	30 979	10 000	92 481
Capital (equipment)					
FRDC TOTAL	**52 007**	**150 073**	**106 894**	**42 000**	**350 974**
Research Organisation contribution (AAHL)	30 857	64 183	35 401		130 441
Research Organisation contribution (DPI Vic)		10 000	11 000	12 000	33 000
Total of Industry & Other Funding	x	x	x	x	x
GRAND TOTAL in $$s					**514 415**

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. Detection of low levels of virus in asymptomatic carriers (e.g. wild abalone broodstock and/or other aquatic animal species) requires the development of these highly specific and sensitive molecular tools such as a quantitative PCR assay.
In addition to surveillance tools, better procedures/reagents for disease diagnosis are required. Presence of histological lesions provides a presumptive diagnosis. The development of in situ hybridisation probe(s) and diagnostic antiserum for the localisation of abalone herpesvirus within histological lesions will provide a means for definitive diagnosis to be made. The development of molecular tools and reagents will allow researchers and industry to rapidly and specifically detect and locate the virus in abalone tissues therefore providing a vital means for diagnosis and facilitating a better understanding of the epidemiology of this disease, leading to more efficient management of disease outbreaks.

Objectives

1. To identify nucleic acid sequences of the emerging abalone herpesvirus (via PCR-based gene amplification and sequencing) necessary for the development of diagnostic tools (e.g. conventional PCR, TaqMan PCR, ISH probe).
2. To develop and validate PCR assays for the detection of the abalone virus.
3. To develop an in situ hybridisation assay specific for the abalone herpesvirus
4. To determine which abalone tissues provide accurate diagnosis of infection.
5. To develop a diagnostic antiserum for use as an immunohistochemical reagent in diagnosis of abalone herpesvirus infections.
6. 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 tests 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.

3. A further output from this project will be a newly qualified diagnostic virologist trained in modern diagnostic techniques that would be suitable for a diagnostic/research position in a State laboratory or research institution.

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 DNA 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 Software version 1.5 (PE Applied Biosystems), primers and probes will be designed to detect the abalone virus.
-Herpesvirus nucleic acid sequences suitable for use as a diagnostic probe will be developed for in situ hybridisation.
-Infection trials of healthy abalone will be performed within the AAHL biosecure facility to determine which abalone tissues are the most appropriate for sampling during an active surveillance program of, for example, wild broodstock.
-Either virus will be purified from host tissue or recombinant virus proteins will be engineered to provide antigens for production of diagnostic polyclonal antiserum in rabbits.
-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
E-MAIL: **joanne.slater@csiro.au**

No later than cob on Friday 23 June 2006.