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Abalone Viral Ganglioneuritis (AVG) Research and Development Needs Workshop

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Abbreviations

AAGA	Australian Abalone Growers Association
ACA	Abalone Council Australia
ACV	Abalone Council Victoria
ACDP	Australian Centre Disease Preparedness
AVG	Abalone Viral Ganglioneuritis
CT	Cycle Threshold
CVO	Chief Veterinary Officer
EMAI	Elizabeth Macarthur Agricultural Institute
eDNA	Environmental DNA
PCR	Polymerase Chain Reaction
WADA	Western Abalone Divers Association

Executive Summary

Introduction

Following Abalone Viral Ganglioneurits (AVG) re-emerging in the Western Zone of the Victorian Abalone Fishery in early May 2021 it was decided that a workshop needed to be convened as soon as possible with relevant stakeholders to evaluate past research and understand research gaps while the virus is active, and samples of active material can be collected and stored in a secure laboratory in readiness for future testing. Stakeholders from wild catch Victoria, South Australia, Tasmania, and New South Wales; farmed abalone sector; government and research agencies and universities gathered for a 1-day workshop, held in Queenscliff on 14th December 2021 to identify and prioritise future research and development priorities.

Background

Abalone Viral Ganglioneuritis (AVG) disease is caused by a herpesvirus (infection with abalone herpesvirus, AbHV) and was first detected in Victoria in December 2005. The last recorded instance of this disease in Victoria was in January 2010 at Cape Otway. The disease was again confirmed in the Cape Nelson area on 2 May 2021. Since then, further detections have been made at several locations and has been confirmed in Victoria as far east as Cape Otway and as far west as the Discovery Bay Marine Park.

While the virus is active there is a need for the Victorian wild catch abalone fishers, abalone aquaculture farms and fishery managers to urgently review past research, to inform a gap analysis and inform further research needs. This review will support those involved in the outbreak and inform fellow members nationally with a summary of past research and a way forward with other research for up-to-date biosecurity protocols, surveillance, and diagnostic tools to aid rapid detection to future proof the industry. Relevant industry, government representatives and stakeholders have been kept informed and asked to comment and contribute issues and ideas for a cooperative structured way forward to build a common framework on which to establish a set of key actions and or priorities.

Aims/objectives

1. Conduct an analysis of past research on AVG, create a plain English summary and identify knowledge and research gaps that can be evaluated nationally by key stakeholders
2. Hold a workshop that will be a mixture of face to face and virtual so that key stakeholders can attend and participate in evaluation of suggested research projects identified
3. Prepare and provide a final report that clearly articulates research priorities for AVG in the short term and long term

Methodology

A key stakeholder workshop was held to progress the project objectives. To inform the workshop discussion, Serge Corbeil (CSIRO) was commissioned at the start of November to review past research papers. A plain English summary of the review of the available research and a Dropbox link to all the relevant papers was provided to attendees prior to the workshop. The review paper was presented at the workshop.

Results/key findings

The forum bought together industry and government participants from Tasmania, South Australia, New South Wales, and Victoria from wild catch and aquaculture as well as researchers and government laboratories and government and private veterinarians and FRDC.

The workshop provided a forum for major stakeholders to discuss past research and identify gaps and urgent activities while the virus remains active. Based on the discussion several research questions were identified and potential research activities scoped, these included:

- Medium term work to look at cost effective and feasible monitoring
- Research about human based transmission vectors
- Study human and animal-based transmission vectors
- External stressors/risks
- Approaches to surveillance – including the potential of eDNA
- Farm based monitoring protocols including multi-jurisdictional support for these protocols to be accepted in the accreditation procedures

It was noted that there is a short window while the virus is active to collect samples for future research. Ideally, this would occur within 14 days of the workshop, just before Christmas. These samples should be frozen for future genotype mapping, PCR testing, assess CT levels, and assessing genetic resistance.

Finally, a series of unknowns and assumptions were discussed. For example, since the virus is transmitted through water, could cray pots, recreational fishers, diving equipment be transmission vectors. This all needs to be validated as user groups are being locked out of an area who may not need to be. Urgent understanding of how the virus moves is required.

Implications for relevant stakeholders

Since holding the workshop Agriculture Victoria and Victoria Fishery Authority (VFA) are progressing an AVG project that will be looking at transition to managing the outbreak, preparation of guidance documents for other zones who have not been infected. This work will also develop protocols for decontamination, chemical recommendations, and usage rates as well as biosecurity risk assessment of current Victorian abalone processors.

Recommendations

It is recommended that immediate collection of samples be collected from uninfected populations, infected populations and previously infected populations and be frozen for future

genetic differentiation studies. Other medium- and long-term priorities be scoped out and supported by the Abalone Council Australia (ACA) or Australian Abalone Growers Association (AAGA) who hold Industry Partnership Agreements (IPA's) with FRDC for project funding.

Keywords
Haliotis rubra, Abalone Viral Ganglioneuritis, biosecurity, surveillance, environmental risk factors, stressors, future proofing, preparedness.

Introduction

Abalone Viral Ganglioneuritis (AVG) occurred between late December 2005 and January 2006 when first mortalities were identified in farmed abalone and subsequently identified in wild abalone stock in the Port Fairy region of Victoria. At the time AVG was considered exotic to Australia but had occurred in Asia. Research results when AVG was discovered, indicated that the virus was highly infectious and capable of causing significant mortalities.

Wild catch, aquaculture and FRDC have invested considerable funds in abalone-related research over the last 17 years with some 80+ projects at a cost of $18.5M[1].

The Table below is an overview of FRDC RD&E investment into wild Abalone between 2002 and 2018 (this is not exhaustive). Total investment ($) has been partitioned into three 5-year periods and is split across several research categories. The per cent of projects in each research category (%) is shown for each 5-year period.

Research expenditure on “Disease/pathogens/biosecurity” has been $4,118,876 and while this is the largest category, during the recent outbreak and reviewing past R&D, more needs to be done to assist industry know more about transmission pathways, what causes the virus to exhibit after such a long time being dormant and better real time detection tools before abalone become infected (wild catch and farmed). Adoption of past R&D projects possibly has not been distilled in a way that will assist the industry and has been aimed more at scientific levels, government regulators and laboratories. In relation to AVG not enough scientific information is available to help gain knowledge from the current outbreak and to have relevant R&D to help deal with any future outbreaks.

[1] Abalone assessment and management: what have we learned, what are the gaps and where can we do better, workshop summary 7 and 8 March 2019.

	2002-2005		2009-2013		2014-2018	
Research Category	**$**	**%**	**$**	**%**	**$**	**%**
Capacity & diver welfare	$334,356	8	$6,000	2		
Centrostephanus	$828,553	8	$483,581	7	$439,712	12
Climate change			$795,163	5		
Community & consumer support			$143,400	5	$168,800	4
Customary issues	$207,395	8			$510,390	4
Disease / pathogens / biosecurity	$344,133	8	$1,280,745	17	$2,493,998	24
General	$426,819	25	$820,451	7		
Markets & trade			$1,164,552	17	$436,500	8
Planning, review & conferences	$20,000	8	$449,844	7	$899,528	16
Recruitment & translocation	$18,500	8	$671,630	12	$887,192	20
Stock assessment & management	$1,081,393	25	$1,823,198	14	$993,606	12
Value adding			$771,070	7		
GRAND TOTAL	$3,261,149		$8,409,633		$6,829,726	

Some research projects carried out, in controlled conditions, since the initial outbreak have concluded:

- Direct contact between abalone is not required for horizontal transmission[2]
- AbHV can survive in the water column at 15°C and remain infectious and cause disease one day after being shed in the water
- Virus survival is reduced at higher temperatures suggesting that transmission is more likely in winter than in summer

Diagnostic techniques were quickly developed after the initial outbreak and a scientific forum was convened in 2006, to discuss R&D requirements in the face of the new emerging disease. (FRDC 2009-032).

Despite all the R&D that has been carried out, there has been no synthesis of the research to provide a "plain English summary" of what has been done to gain an understanding of gaps, diagnostics and ongoing surveillance required to understand outbreaks.

The outbreak early May 2021, it was speculated by authorities to have occurred due to an extreme weather event that may have caused stress on the abalone triggering the re-emergence of AVG. The weather event has not been scientifically proven as the reason for the virus to emerge after being dormant since 2010.

It is not well understood how the virus spreads and if human activity assists in the movement. VIC Agriculture were very quick to enact Movement Control Orders as soon as the May outbreak was known. This order restricted other commercial operators and recreational fishers from

[2] Abalone herpes virus stability in sea water and susceptibility to chemical disinfectants Serge Corbeil ∗, Lynette M. Williams, Jemma Bergfeld, Mark St.J. Crane. Nov 2011.

using the area of the outbreak to limit the spread. Commercial fishers, such as rock lobster, were prohibited from fishing while the Movement Control Order was in place as it is not understood if dragging lobster gear on the ocean floor helped to move and transmit the virus to more areas.

It was decided that while the virus was active it was critical to hold and AVG R&D needs workshop to understand past research and the gaps and to develop a set of R&D priorities that could be carried out in the short and long term. The workshop would include key stakeholders across Australia including wild catch fisheries, aquaculture, government agencies, researchers, and recreational fishers.

Objectives

The project objectives were to:

1. Conduct an analysis of past research on AVG, create a plain English summary and identify knowledge and research gaps that can be evaluated nationally by key stakeholders.

2. Hold a workshop that will be a mixture of face to face and virtual to enable key stakeholders to attend, participate and contribute to identifying potential projects.

3. Prepare and provide a final report that clearly articulates research priorities for AVG in the short term and long term.

Method

Review of Abalone Viral Ganglioneuritis research

A review of past AVG research was conducted by CSIRO, Serge Corbeil (Experimental Scientist, Fish Disease Lab, Australian Centre for Disease Preparedness [ACDP], CSIRO) that was carried out before the workshop to inform the discussions on the day. Serge provided a list of and access to research papers summarising their research findings. These were shared with participants prior to the workshop. The pre workshop reading material contained 13 key observations from the past R&D analysis together with some pros and cons.

Dropbox link to papers:
https://www.dropbox.com/sh/3ll2qpffirmej8l/AAAxInzOQ2ENBgFsSUMPcmvwa?dl=0

The list of papers used in the review are shown towards the end of this report under **References**.

Stakeholder workshop

An invitation via email was distributed to key stakeholders including – wild catch, farmed, government agencies, researchers, veterinarians, recreational, rock lobster, South Australia, New South Wales, and Abalone Council Australia.

The intent of the workshop was to bring together key stakeholders to evaluate past research and identify research and knowledge gaps for AVG since the initial outbreak in 2005, the disease has been dormant since 2010 and re-emerged in 2021 why and how is unknown and while it is speculated that a stressful weather event triggered the recent outbreak it remains a speculation from assumptions not research findings.

Research carried out post the 2005 outbreak revealed that the disease can be transmitted horizontally, the virus was transmitted through the water column and direct contact was not required for transmission, the virus remained virulent and pathogenic after being frozen at 80°C.

Unknown still from the 2005 outbreak: host range, distribution and prevalence, infectious dose, routes of transmission, control methods and similarity to the Taiwanese virus.

During the most recent outbreak a full emergency response was mounted by Agriculture VIC biosecurity team, the Chief Veterinary Officer in conjunction with the VFA and a Movement Control Order was quickly established limiting entry into the area for other commercial and recreational fishing operators. The abalone virus control area was lifted on 12th October 2021, and those involved are wanting to understand more about this virus while the virus is active, from the unknown issues listed above questions remain unanswered including, but not limited to:

- What is the distribution and prevalence particularly on gear such as wet suits, dive gear, bags, bins, tools, gloves?
- Is AVG transmissible on lobster pots and ropes or recreational fishing gear?
- What is the best treatment for bins that have been in contact with infected abalone -what are the risks?
- Can frozen “abalone snot/mucus” be tested and used for transmissibility trials?

These are considered critical knowledge gaps and agreed by Abalone Council Australia, Abalone Council Victoria members, NSW abalone divers, government agencies and researchers. These various groups have all agreed that an analysis of past research, development of a plain English summary as well as identify research and knowledge gaps is critical now that AVG is no longer considered as exotic to Southwest Victoria.

Stakeholder consultation

Abalone Council Victoria (ACV) has council members covering all three Victorian wild catch abalone zones including the processors. ACV consulted with Agriculture VIC and VFA through all the AVG response and preparation for this project review and the workshop. Western Zone members are at the “coal face” of this disease and their input has been valuable in contributing to this AVG project, workshop and collecting abalone samples in readiness for future research projects.

Working closely with Agriculture VIC and VFA was critical to being informed on the response activities and as the response transitioned into Phase 2 Response. Western Zone through the involvement of Harry Peeters and Craig Fox were instrumental in obtaining abalone and water samples identified as a need during the workshop. Appropriate permits were obtained to collect these samples, which have been frozen and await future R&D projects.

Results/Discussion

Pre-workshop reading material – Abalone Viral Ganglioneuritis: An update by Serge Corbeil, Australian Centre for Disease Preparedness (ACDP)

The purpose of the review was to provide the Australian abalone industries, as well as other stakeholders, updated information on the current knowledge of AVG in Australia and possible research avenues to reduce the gaps in knowledge for this disease. Readers are encouraged to read the main AVG review published by Corbeil (2020) for supplementary information. (http://doi:10.3390/pathogens9090720)

Note that the original virus name abalone herpesvirus 1 (AbHV-1) has been changed to haliotid herpesvirus 1 (HaHV-1) by the International Committee on Taxonomy of Viruses to adhere to official taxonomic classification requirements.

Abalone Viral Ganglioneuritis (AVG) diagnostic tests currently available in Australia:
The Corbeil (2020) AVG review provides more detailed information on some of these assays.

Histopathology and electron microscopy:

- **Pro**: Provides general diagnosis of viral infection but limited to investigation of clinical disease. Not a specific surveillance test due to lack of sensitivity in apparently healthy animals
- **Cons**: They are time consuming and moderately costly to perform.

Conventional PCR assays:

- **Pros**: Provide sensitive and specific diagnosis, when amplicons are sequenced. Primarily used for confirmatory diagnosis of samples screening positive by real-time PCR assays. Allows partial gene sequencing and genotype identification (e.g. Vic-1, Tas-1, Tas-2, Tas-3, Tas-4, Tas-5, Taiwan, China). Assays described in the OIE Aquatic Manual for infection with abalone herpesvirus.
- **Cons**: Specialised laboratory equipment and technical knowledge are required to perform the tests. Contamination can occur resulting in false positive results (however, sequencing allows for confirmation of results). Moderately costly.

Real-time PCR assays (qPCR):

- **Pros**: Provide a very rapid, highly sensitive and specific detection. Suited to high-throughput screening for investigation of clinical disease and surveillance in apparently healthy animals. Validated assays described in the OIE Aquatic Manual for infection with abalone herpesvirus.

- **Cons:** Specialised laboratory equipment and technical knowledge are required to perform the tests and interpret the results. Do not provide virus strain identification. Moderately costly.

In situ hybridisation (ISH):

- **Pro**: Provides information on the virus location in the abalone tissues. Confirmatory diagnostic test for suspect positives identified by histology.

- **Cons**: Time consuming and moderately costly. Specialised laboratory equipment and technical knowledge are required to perform the tests and interpret the results. Does not provide virus strain identification.

Existing AVG diagnostic test not yet available in Australia

Loop-mediated isothermal amplification procedure (LAMP):
An AVG (LAMP) procedure was developed by Chen et. al. (2014) to amplify HaHV-1 DNA with high specificity, sensitivity and rapidity under isothermal conditions.

- **Pros:** Provides very rapid, sensitive and specific detection. Can be used outside sophisticated laboratory and requires less technical knowledge. Cheaper than tests described previously.
- **Con**: Does not provide virus sequences for strain identification. Very limited published performance data.

Potentially useful AVG diagnostic tests (not yet developed for AVG)

Rapid Diagnostic Test (RDT):
RDTs are designed for use at the point of care/pond side and can be adapted for use in low-resource settings. An RDT is low cost, simple to operate and read, specific, stable at high temperatures, and works in a short period of time. Depending in the format, RDTs may be used for screening of clinically diseased animals or surveillance of apparently healthy animals. No RDTs for aquatic animal pathogens have been authorised for us in Australia. AFDL has a current FRDC project (FRDC 2019-089) evaluating chromatographic strip and PCR point-of-care tests for detection of WSSV.

Antibody-based lateral flow test and flow-through tests would contain HaHV-1 specific antibodies and show a colorimetric reaction if the virus is present in the sample (like a pregnancy test) (Rapid Diagnostic Test (RDT) (emory.edu).

A different approach to this technology is called the Recombinase Polymerase Amplification Lateral Flow Dipstick (RPA-LFD). It is based on DNA amplification under isothermal conditions. The product (amplified viral DNA) produced by the RPA is then easily detected by a semi-quantitative colorimetric change on the LFD strip.

- **Pros**: Rapid and specific. Does not require a costly equipment. Results can be obtained within a short period of time (3 to 5 minutes for lateral and flow-through tests, up to 40

minutes for the DNA test). Useful for in field application. The test could be as sensitive as the nested PCR (laboratory based test) (Jaroenram and Owens, 2014).

- **Con**: Does not provide virus sequences for strain identification. Depending on format may be limited to use for screening clinically diseased animals due to sensitivity, may require specialist training and increase risk of false-positive results due to contamination with positive control material. No RDTs to detect aquatic animal pathogens are approved for use within Australia.

Detection of environmental viral DNA in sea water

Flow cytometry:

The flow cytometry test for the oyster herpesvirus (OsHV-1) was developed (Flinders University in collaboration with the South Australian Research Development Institute (SARDI) Aquatic Sciences) using an off the shelf entry level flow cytometer and targeted seawater and/or oyster tissue test matrices. After developing OsHV-1 specific reagents and protocols the Merck Muse flow cytometer (Muse) successfully detected and quantified OsHV-1 in sea water samples (Mitchell and Paterson, 2019).

- **Pros**: According to the research groups it is a low cost ($2.00 per sample) and quantitative test (limit of detection 14 OsHV-1 particles per mL). Can be run in the field or at the hatchery by a trained technical officer. Could be developed for HaHV1.
- **Con**: Require regular maintenance by a trained technical officer? Emerging detection technology for aquatic animal pathogens

qPCR (TaqMan):

Same qPCR technique used for detecting and quantifying HaHV-1 DNA in abalone tissues, the difference being that viral DNA is extracted from sea water or plankton surrounding abalone colonies rather than from abalone tissues.

- **Pros**: Could help foresee eventual disease outbreak if performed regularly. Sampling water in areas where no moribund/dead abalone are located (e.g. lobster fishing areas) could help evaluate/mitigate the risks of HaHV-1 transmissions by divers harvesting their catch and setting up their cages in other locations. DAWE-funded work done by AFDL and SARDI for detection of WSSV and OsHV-1 in plankton.
- **Con:** Requires extra work and material to collect and filter sea water/plankton samples and extract the viral DNA.

MinION:

Field-portable whole genome sequencing platform. Emerging technology for eDNA detection.
AFDL has a current FRDC project (FRDC 2018-147)
evaluating MinION including comparison with existing established diagnostic assays.

- **Pros:** Rapid.
- **Cons**: Unknown sensitivity and requires expert interpretation of data to avoid false-positive results.

Abalone tissue types for diagnostic activities

The abalone tissues affected by HaHV-1 (Chinese variant) include both neural tissue and hemocytes (Bay et al., 2019). A more recent study by the same authors (Bay et al., 2020) showed that the same Chinese variant of HaHV-1 can also be detected in the mantle, gills and the hepatopancreas.

To date no studies has investigated the distribution of Australian HaHV-1 variants in abalone tissues besides neural tissues. Due to the similarity between Chinese and Australian variants it is possible that the tissue distribution is similar. A study would need to be performed to confirm this. Additional samples to evaluate include haemolymph and swabs which may enable evaluation of the effect of pooling to reduce test costs, without compromising test sensitivity.

Comparison of Ct values between original AVG outbreak in 2006 and current outbreak in 2020-21

Samples from the original outbreak were initially tested by histology and conventional PCRs (presence or absence of DNA bands on gel but no Ct values). Later on qPCRs (ORF-49/66/77) were developed (2008+) and subsequently validated using a small number of positive and a large number of negative fixed samples. A direct comparisons of Ct values between the outbreak events would not be an accurate indicator of differences between virus pathogenicity (ability to cause disease and mortality in infected abalone). Many different factors can contribute to different levels of pathogenicity for the same virus isolate. For example, life stage of abalone, abalone population/genetic diversity, abalone immune status, environmental conditions (e.g. water temperature, other stressors etc.), laboratory equipment, technician etc.).

Even though a mortality rate in the field is subject to similar limitations, mortality rate would probably be a more accurate indicator of the severity of outbreaks than qPCR Ct values.

CT values and their relationship to AVG prevalence and infectivity

Recent Covid-19 outbreaks and their impacts on human health and economic activities have led scientists to investigate the relationship between the qPCR diagnostic tests (which provide specific Ct values) and the probability of transmission and infection to non-infected people (Kampf et al. 2020).

Such correlation has not been evaluated for AVG in abalone. To date the HaHV-1 qPCR assays have been used to determine the presence of HaHV-1 infection in abalone, demonstrate freedom from HaHV-1 in population of abalone and to certify individual abalone free from HaHV-1 for trade or movement purposes (Caraguel et al., 2019). However, it would be possible, through laboratory transmission trials, to quantify a HaHV-1 stock solution using qPCR and establish its Ct value. Subsequently, serial dilutions can be performed and expose naïve abalone to this series of virus dilutions, simulating exposure of abalone to virus by horizontal transmission. Mortality rates for each dilution as well as presence and quantity of virus in sub-clinical/uninfected animals could be established. This would allow estimation of the virus concentration (via its Ct value) per mL of water necessary to induce an active infection in abalone. However, this would not provide information on an already sub-clinical infection escalating to clinical disease.

There are limitations of such information and extrapolation to the 'real-world' of abalone reef or land-based abalone farms. Firstly, the Ct value is based on the presence of HaHV-1 DNA in any given sample which is not indicative of infectious viral particles, therefore is likely to overestimate the infectious virus quantity.

Secondly, at ACDP challenge trials are performed under static conditions (water remaining in the tanks in the presence of the abalone without being flushed) which does not represent the dynamic water flow in the ocean and most farms.

Thirdly, the time frame of exposure is limited in the laboratory and would not provide an accurate representation of longer time frame and constant virus shedding (at low Ct values by sub-clinically infected abalone) and its potential effects on close by naïve abalone in the reef or the farms through continuous exposure.

With regards to quantifying the amount of virus (using qPCR Ct values) in abalone testing positive (as opposed to testing the water), it is not known how much virus is shed by these animals. Once again evaluating it in the laboratory would provide data that are unlikely to be representative of the ocean and farms setting.

To date the severity of AVG outbreaks has been mainly evaluated using the morbidity/mortality rates rather than qPCR Ct values. However, when we 'interpret' Ct values we generally use the subjective ranges: 1 to 20 very high; 21 to 25 high; 26 to 30 moderate, 30 to 35 low, 35 to 40 (or 40+) very low (potentially sub-clinical carrier).

Current outbreak (2021) HaHV-1 partial genes sequencing

Viral DNA isolated from sick abalone harvested from the infected Victorian waters in 2021 identified the variant as Vic-1 with a 100% nucleotide sequence identity with the original 2006 variant causing the first AVG outbreak (c.f. Table 1a and 1b) (data obtained from ACDP Fish Diseases Laboratory). Two different partial gene segments (1213 and 1617 fragments) were used for the analyses. The full viral genome sequencing has been performed by Agribio laboratory Victoria and will be available shortly.

Table 1a

	HaHV-1 (2021) % nucleotide identity within the 1213 gene sequence
HaHV VIC1	100
HaHV TAS1	94.3
HaHV TAS2	88.1
HaHV TAS3	89.1
HaHV TAS4	87.6
HaHV TAS5	95.1
HaHV Taiwan2004	87.2

Table 1b

	HaHV-1 (2021) % nucleotide identity within the 1617 gene sequene
HaHV VIC-1	100
HaHV TAS-1	87.0
HaHV TAS-2	94.0
HaHV TAS-3	92.0
HaHV TAS-4	89.6
HaHV TAS-5	94.2
HaHV Taiwan2004	92.0

HaHv-1 Transmission

Although a preliminary experiment (Corbeil et al., 2014) suggested that mucus did not confer protection and longer survivability to HaHV-1 in sea water, a fully validated study (including several replicated from many abalone sources) was not conducted. This is due to the difficulty in inducing mucus excretion in infected abalone in a large amount in laboratory conditions. A larger scale experimental study would need to be performed to establish indubitably the protective property of abalone mucus to the virus. With regards to the virus survivability on wetsuit, cages, ropes out of the water, no information is available at this time. This topic would need to be investigated.

Disinfection methods

HaHV-1 infectious water directly treated with calcium hypochlorite at 1.5 and 2.0 ppm of residual free chlorine (initial concentration was 10-15 ppm) were sufficient to inactivate the virus during a 15 min exposure (viral titre 1.67×106 gene copy/mL) in clean filtered sea water (Corbeil et al., 2012).

In a study on OsHV-1 (structurally similar herpesvirus of oyster) Hick et al. (2016) successfully inactivated infectious water using a dose of sodium hypochlorite calculated to provide 50 ppm residual free chloring in relatively clean sea water (Hick et al., 2016). Addition of organic matter to the water reduce the efficacy of chlorine to inactivate the virus. In addition, in a study on carp, Kasai et al. (2005) recommend 3 ppm of residual free chlorine for a 20-minute exposure to inactivate Cyprinid herpesvirus 3 (CyHV-3).

Hicks study on OsHV-1 showed that a 15-minute treatment of 1% (weight/volume) Virkon-S was effective at inactivating the virus. Heat treatment at 50°C for 5 minutes was also sufficient to inactivate OsHV-1. Because OsHV-1 and HaHV-1 are closely related it is highly probable that these treatments would also inactivate HaHV-1, however, only reproducing these experiments would confirm this assumption.

Virucidal efficacy of iodophors such as Buffodine and the non-iconic surfactant Impress are very effective at inactivating HaHV-1 in water (Corbeil et al., 2012). As for OsHV-1, 0.1% iodine for 5 minutes was effective at inactivating it, however, an alkaline detergent was not effective (Hick et al., 2016). When OsHV-1 is present in homogenised oyster tissues Hick's study showed that OsHV-

1 inactivation was successful with the use of either Virkon-S, a quaternary ammonium compound, sodium hydroxide, formalin, or iodine. However, sodium hypochlorite (100-200 ppm residual free chlorine) was not effective.

Of note, any virucidal agents (including those tested in the previously mentioned studies or already used in the field (e.g. iodine, formalin, sodium/calcium hydroxide, Virkon-S, Virukill) are described as hazardous requiring protective equipment and/or are toxic to aquatic life therefore not to be used near streams, lakes, ocean etc. (Material Safety Data Sheets available on-line).

Focusing on the land-based farms (recirculating aquaculture system) Camilla Martins (Yumbah Aquaculture) developed an extensive methodology to test the impact of various chemical treatments on the abalone's health, behaviour, and survival. Although AVG was not part of her study it is important to consider the effects of the chemical on abalone when developing herpesviruses disinfection protocols. Table 3 presents the list of chemicals and levels of residual produced.

The abalone were exposed for 60 minutes to the chemicals through bathing with subsequent monitoring in clean water for 15 days.

Camilla concludes her report stating that the non-ionic surfactant EnviroClean should not be tested in future trials as it caused 100% abalone mortality. The chlorine dioxide *Zydox* should be tested in future trials but at lower concentrations and/or shorter exposure time. Calcium hypochlorite, hydrogen peroxide and the iodophors *Buffodine* and *Ovadine* should also be tested in future trials.

Some considerations from the information in her report are:

- Calcium hypochlorite and Buffodine can inactivate HaHV-1 but there is not information on the other chemicals against this virus
- The chemical has to be approved by APVMA to be used on abalone. Currently there is an APVAMA permit (PER83276) for hydrogen peroxide 60% to be used in the treatment of metazoan and protozoan ecto-parasitic infestations and the control of fungal infections in freshwater and saltwater finfish and finfish eggs. The bath treatment can be up to 60 minutes and the withholding period is nil
- A chemical with short or no withholding period is desirable
- Organic matter inactivates a number of chemical disinfectants
- Hydrogen peroxide can harm biofilter in a recirculating aquaculture system
- Ovadine is more environment friendly than Buffodine because the latter has a non-ionic surfactant in its composition

Further information can be obtained from her report (Martins 2018).

Table 2 Chemical treatments for aquatic species permitted by the government.

Chemical name/group	Permit	Dosage (details on permit)	Research info
Chlorine (EEZI pool 650g/kg as calcium hypochlorite)	PER8206	200ppm	Corbeil et al. (2012) Calcium hypochlorite (Sigma-Aldrich) **Injection trial** Groups of 6 abalone were injected intramuscularly with 100µL of treated virus (20×10^6 viral gene copies). This viral titre is approximately 100,000 times higher by injection. **Injection trial #1** Low dose 5ppm (100% mortality) Medium dose 10ppm (66% mortality) Immersion trial Infectious water containing 1.67 x 10^6 viral gene copies/ml was treated with calcium hypochlorite to a final concentration of 10 and 15ppm. 100ml of treated water was then added to 900ml of seawater for immersion challenge. This viral titre was more than 100 times higher that is required to cause 100% mortality after immersion challenge Medium does 10ppm (0% mortality) Medium dose 15ppm (0% mortality)
Agridyne (iodines 16g.l	PER8206	0.1% solution	
Detscan (Quart ammonium 25g/l	PER8206	0.2% solution	
F1OSC (Bensalkonuium chloride 54g/l and poly hydrochloride 4g/l)	PER8206	0.2% solution	Corbeil et al. (2012) Impress disinfectant Low dose 1% High dose 5% Bath 1% and 5% effective at the inactivation of virus at 20 x 10^6 viral gene copies via injection (tested on 6 abalone). NOTE: 20 x 10^6 viral gene copies. This virus titre is approximately 100,00 times higher than that required to kill an abalone by injection.

- Obtained from the AVG Decontamination Working Group.

Table 3 Chemicals and their residual

Bath treatment	Residual chemical	Level of residual chemical
Non-ionic surfactant EnviroClean 1%	Benzalkonuium chloride	~ 250 ppm
Calcium hypochlorite 15 ppm Calcium hypochlorite 25 ppm	Free chlorine Free chlorine	~ 10 ppm ~ 17 ppm
Iodophor *Buffodine* 50 ppm Iodophor *Buffodine* 150 ppm Iodophor *Buffodine* 250 ppm	Iodine Iodine iodine	~ 1 ppm ~ 3 ppm ~ 5 ppm
Iodophor *Ovadine* 50 ppm Iodophor *Ovadine* 150 ppm	Iodine iodine	~ 0.5 ppm ~ 1.5 ppm
Chlorine dioxide *Zydox* 25 ppm Chlorine dioxide *Zydox* 50 ppm	Chlorine dioxide Chlorine dioxide	~ 25 ppm ~ 50 ppm
Hydrogen peroxide 50% 25 ppm Hydrogen peroxide 50% 50 ppm	Hydrogen peroxide Hydrogen peroxide	~ 25 ppm ~ 50 ppm

- Obtained from Camilla's AAGA (2018) presentation

Table 4 Summary of disinfectants used by the industry

Chemical name/group	Permit	Dosage (details on permit)	Comment
Virukill (dodecyldimethyl ammonium chloride)			Hazardous, corrosive
Virkon aquatic			Kills crustaceans etc.
Chloring (EEZI pool 650g/kg as calcium hypochlorite)	PER8206	200 ppm	
Agridyne (iodine 16g/l)	PER8206	0.1% solution	
Detscan (Quartammonium 25g/l)	PER8206	0.2% solution	
F10SC (Benzalkonuium chloride 54g/l and poly hydrochloride 4g/l)	PER8206	0.2% solution	
Truck wash (sodium dodecylbenzene sulfonate)			

- Obtained from the AVG Decontamination Working Group

Ozone and U.V. Treatments

In his thesis on the epidemiology of AVG, Ellard (2015) specified that the presence of water treatments such as ozone or UV are considered to be protective and may play an important role in keeping circulating levels of virus to a minimum, which in turn delays spread of disease. When

used in closed systems, circulating ozone levels must not impact on bacteria within biofilters, thus its purpose is to reduce levels of circulating pathogens and cannot eliminate them completely.

Abalone industry biosecurity practices in Australian States

In commercial abalone diving, different practices occur across Australia ranging from no biosecurity al all to treatment of gear and boats with a number of disinfectants. In many cases, the efficacy of these disinfectants against the virus has not been assessed.

Listed below are Australian States on-line resources on biosecurity to reduce the risks of AVG transmission:

- Abalone disease | Animal diseases | Biosecurity | Agriculture Victoria
- Abalone Disease - VFA
- Microsoft Word - English Abalone disease fact sheet FINAL 230621 (vfa.vic.gov.au)
- Abalone viral ganglioneuritis (AVG) (nsw.gov.au)
- Standard operating procedures: decontamination for commercial abalone divers (nsw.gov.au)
- Commercial Abalone Fishing Biosecurity Measures | Department of Natural Resources and Environment Tasmania (nre.tas.gov.au)
- Guidelines and Resources - DAWE
- Abalone viral ganglioneuritis - PIRSA

AVG Expression in abalone and HaHV-1 carrier state

In Ellard's thesis (2015), the author suggested that based on observations made during the course of AVG outbreaks in Tasmanian abalone processing plants, causal factors for acute AVG differs from that of the observed chronic form of AVG. In the acute form, exposure of naive abalone cohorts to HaHV-1 or non-endemic stains of virus appears to be the principal cause of disease. This most commonly occurs when separate populations are mixed but can occur through carriage of the virus in water or where abalone are placed into live-holding systems contaminated with high viral loads. In the case of chronic AVG, this is considered to express as disease when abalone, already infected with an endemic strain of HaHV-1 prior to entering live-holding tanks, are then exposed to environmental stressors resulting in shedding of the virus into the environment resulting in clinical disease.

With regards to the different disease expression in different Blacklip Abalone populations Ellard hypothesised that abalone are sedentary animals that tend to remain on the same reef system throughout their life. As a result, genetic mixing between specific reef populations is limited. 15 Tasmanian abalone populations have been shown to be genetically distinct from mainland populations. In addition, Blacklip Abalone populations located in different regions of Tasmania have evolved according to local evolutionary pressures. One of these evolutionary pressures could be an ability to tolerate the presence of HaHV-1.

AVG resistance in abalone and management applications

Further in his thesis, Ellard addressed the AVG resistance topic, here is verbatim: 'Previous research suggests that abalone do not develop acquired immunity to HaHV-1 infection post exposure (Corbeil et. al., 2014). Molluscs, including abalone, rely on an innate immune system mediated by haemocytes, but no adaptive immune system is known to occur (Magnadottir, 2006). Instead, it is suggested that local evolutionary pressures result in the development of innate resistance over time. This view is supported by studies of isolated abalone populations in California that were decimated by the disease Withering Syndrome. Remnant black abalone populations demonstrated resistance, but this resistance was not associated with increased ability to inactivate and eliminate the pathogen. Instead, an ability to tolerate presence of the pathogen was observed in recovered populations. It was suggested that immunity to Withering Syndrome is, in part, a result of host tolerance of infection (Freidman et. al., 2014)'.

A more recent study on Victorian Blacklip Abalone resistance was conducted by Miller et al. (2021) who carried out a full genome characterisation of blacklip abalone from 14 Victorian fishing stocks varying in historical exposure to AVG. They found gene patterns of divergent adaptation, some of these gene patterns are known to be associated with HaHV-1 immunity in the New Zealand Päua (*Haliotis iris*) which is resistant to AVG (Corbeil et al., 2017; Corbeil and Helbig, 2019). Miller's project outputs have management applications and clear pathway to adoption by the wild fisheries sector such as translocation of abalone with AVG resistant genotypes to various regions where the genotypes are absent as well as the farm sector by developing selective breeding programs. However, before committing to such applications the next step is to perform quantitative experiments (e.g. laboratory experimental challenge trials) to validate if, and how much, resistance to HaHV-1 is determined by the genotypes identified in the genomic study and if heritability of the resistance trait exist.

"Molecular vaccination'

The 2020 AVG review presented some information on general immune-stimulation of oyster to combat OsHV-1. The same strategy was recently used by Karla Helbig (LaTrobe University) to evaluate its efficacy against HaHV-1 in hybrid abalone. Results from this preliminary experiment indicated that 16 abalone injected with immune-stimulants were 100% protected against HaHV-1 challenge while control abalone had a 100% mortality rate (personal communication). Although in the very early stages, this is an interesting research area that could potentially benefit the farm sector. However further experimentation to clarify dose, exposure method and duration need to be undertaken.

Stakeholder R&D Workshop

The Chair, Dr Ian Knuckey, opened the workshop, outlining the purpose and objectives for the day. From the development of the pre-reading material, it was determined that the key future R&D areas related to three key areas:

1. Detection and identification techniques
2. AVG Monitoring (wild stock and farmed)
3. Reducing transmission and infection (wild stock and farmed)

Against these three focus areas, the workshop participants discussed potential R&D needs and partners that were needed to address the impacts of this AVG event and better safeguard the abalone resource from future events. This included explore key information gaps on the transmission of the virus in the wild.

Project concepts that came out of the workshop are outlined below, please note the numbering does not represent R&D priority just a list of possible activities that can be developed as expressions of interest and put forward to FRDC through respective industry IPAs or funded by other possible means for example Victoria Agriculture/VFA AVG project management.

1. **Genetic survey**
    - Within 14 days post the workshop (before Christmas 2021) - Immediate collection, of abalone from uninfected populations, current infected populations, and previously infected populations. These should be frozen for future genotype mapping, PCR testing, assess CT levels and assess any resistance genes. Collection from each site will need to include GPS co-ordinates.
    - Samples to be collected from uninfected populations at Julia Percy, Discovery Bay, Julia Bank and Cape Otway – 25 randomly selected abalone per site.
    - Samples to be collected from known infected populations at Yellow Rock, Water Springs and Blowholes – 50 random moribund abalone from sites plus water samples and 50 random healthy abalone from each site.
    - Samples to be collected from previously infected populations at Port Fairy, Crags, Water Tower and Leavies – 25 randomly collected per site.

Status	Reef	# of samples
uninfected	Julia Percy	25
uninfected	Discovery Bay	25
uninfected	Julia Bank	25
uninfected	Cape Otway	25
infected	Yellow Rock	50
infected	Water Springs	50
infected	Blowholes	50
prev infected	Port Fairy	25
prev infected	Crags	25
prev infected	Water Toer	25
prev infected	Leavies	25
	TOTAL	**350**

    - Abalone samples collected are to be kept frozen at –20°C at VFA facilities, or other suitable secure freezer area, in readiness for future research proposals.
    - **NOTE** a research permit will be required before this can be commenced.
    - Potential for collecting water samples at the same time above abalone samples are collected, using enviro eDNA collection sampling kits and holding for future possible

research projects and analysis. Potential to evaluate if wetsuits etc carry the virus when in a “clean” zone.

- Recent paper from caged fish in Mediterranean suggested eDNA could detect virus rise ~ 5 days prior to onset of mortality. The practicality of high frequency testing of many sites looks challenging. The difficulty of handling false positives also likely to be challenging. https://pubmed.ncbi.nlm.nih.gov/34704786/
- **Objectives**: to conduct genetic analysis from various populations in the Western Zone to evaluate if survivors have avoided coming into contact with the virus or if they are genetically distant and potentially resistant to AVG.

2. **Assessing cost effective and feasible monitoring**
    - **Objectives**: Preparation for future outbreaks, look to develop tools that provide evidence of environmental, stressors or other factors that might contribute to an outbreak occurring.

3. **Research for human-based transmission vectors**
    - **Objectives**: To develop nationally agreed protocols for vessel decontamination and disinfecting gear that has come into contact with the virus or been used in an area where the virus is active. Develop simple effective fit for purpose protocols using a risk-based approach that can easily be adopted by user groups who are active in areas of the virus.
    - Update the 2009 Biosecurity Control Measures for Abalone Viral Ganglioneuritis: A Code of Practice. Prepared as part of FRDC project 2006/243 by Gavine, F.M., Ingram, B.A., Hardy-Smith, P., and Doroudi, M.
      https://www.vgls.vic.gov.au/client/en_AU/search/asset/1012979/0
    - **NOTE:** This priority has some overlap with the current work of the AgVic/VFA AVG Project and the Code of Practice will be updated as part of the project. An AVG decontamination working group has been established through the Sub-committee on Aquatic Animal Health and may undertake some of the identified objectives.

4. **Study human-based transmission vectors before end of February 2022**
    - **Objectives**: Current control measures restrict certain groups from entering a known infected site and since the virus is transmitted through the water column it could adhere to gear that has been used in the virus infected areas such as cray pots and ropes, recreational fishers’ equipment, diving equipment, wet suits, diving gloves, abalone removal tool to name a few.
    - Carry out a qualitative risk-assessment of human based AVG transmission vectors through an expert elicitation process and from this conduct laboratory trials of each high-risk transmission vector to determine if it supports transmission.
      For those proven transmission vectors, carry out trials of intervention methods to inhibit transmission by collaborating with industry to assess the feasible/cost effective intervention methods identified possibly using UV, fresh water, chemicals, air drying etc or other management protocols.
      Provide a report on results to inform future biosecurity protocols.

- Areas that may be explored - can a wet suit, for example, that has come into contact with the virus infect healthy abalone populations? Is locking out certain groups with a control order valid?
- To understand what risk abalone bins pose in moving the virus to healthy zones and states that do not have the virus and to understand how long the virus remains active out of the water on any of these items; will fresh air, or fresh water, kill the virus?

5. **External stressors/risks environmental (physical/biological)**
   - It was speculated that a terrible weather event before the May 2021 AVG outbreak could have been the stress event that triggered the re-emergence of AVG in the Western Zone, which had been dormant for over ten years. Ongoing investigations by AG VIC/VFA have concluded that this may not have been correct. A study of environmental changes that may have contributed to stressing the abalone could be valuable. NSW for example undertake value mapping of the marine estate which identifies user groups and activities that take place.
   - **Objectives**: To understand physical and biological short term and long-term changes in the environment, temperatures, pesticides or other run off, seismic activity, any other marine estate user groups that are undertaking a new activity that previously did not exist. In carrying out any review stressors should align with previous and current outbreaks.
   - Dr Matt Landos was able to provide several links as evidence of chemical pollution impacting fisheries. www.ipen.org/news/chemical-pollution-causes-fish-declines
   - The above report provides referenced science about the myriad forms of pollution that are impacting nearshore habitats most directly and having impacts on fisheries. The report seeks to expand people's understanding that declines in fisheries are not solely due to overfishing.
   - The two links below show forestry pesticides from plantation forestry end up in waterways and that when bivalves are exposed over long periods it is detrimental to their health:
     - https://phys.org/news/2021-03-explores-link-forestry-pesticides-aquatic.html
     - https://phys.org/news/2021-12-long-term-exposure-environmentally-relevant-pesticides.html

6. **Surveillance of high-risk vectors at opening of closures**
   - This will be managed by current AG VIC/VFA joint AVG response/project management in collaboration with WADA.
   - **Objectives:** Continue ongoing surveillance and disease monitoring to track its movement. Prepare guidance documents for Victorian Central and Eastern Zones for actions to be undertaken if the virus is detected beyond the current areas. Other states and farms need to be prepared in case the virus does not die out as predicted but continues to spread.

7. **Potential of eDNA to detect AVG outbreaks**
   - Genics and Deakin University through Adam Miller have provided the following breakdown for the potential of eDNA for AVG.

- **NOTE:** water samples will be collected for this purpose during field trips to gather wild abalone for project concept number 1.
- **Mapping AVG outbreaks in the field:** Develop an eDNA detection program allowing for the rapid detection of AVG from water samples sourced from the wild and mapping the spread of the virus. Environmental DNA technologies allow for the rapid detection of species via the presence of their genetic material in the environment. The qPCR assay currently used to detect AVG infections in abalone specimens could be translated to AVG eDNA applications quite easily. We propose to fit our commercial fishing vessels with 'citizen science eDNA samplers' that will allow for rapid field sampling and preservation of eDNA directly from and around sites impacted by AVG, and sites at risk of infection. In the short term this assay could assist the wild fisheries in characterising the disease front and linking virus spread to natural processes and commercial activities.
- **Objectives:** To analyse water samples from the current outbreak, develop protocols for water sampling and storage ensuring stopping cross contamination of abalone to water. From this assess the feasibility of using eDNA to monitor future outbreaks.

8. **Farm based monitoring protocols**
    - **Objectives**: To improve the reliability of detection and to evaluate the most cost effective and efficient options for rapid disease detection/onsite/cheap that are accurate and approved by the Commonwealth and states through Animal Health Committee as reliable tools.

Conclusions

Stakeholders involved in the workshop agreed that there were eight research concepts that would be beneficial to assist with detection, identification, monitoring and reducing transmission. Of particular interest and concern was lack of knowledge around human-based transmission via fishing vessels, fishing equipment used by both other commercial fishers and recreational fishers as well as diving gear and bins used by abalone divers. Rapid detection tools were also identified as being needed for both the wild catch and farm sectors.

Summary of the eight research activities:

1. Genetic survey of AVG from collected abalone samples
2. Cost effective and feasible monitoring
3. Research and protocols for vessel decontamination and disinfecting gear that has been in direct contact with the virus or in areas where the virus has been active
4. Study human-based transmission vectors, risk assessments and biosecurity protocols
5. Understanding external stressors/environmental risks to better understand causes of AVG emergence
6. Surveillance of high-risk vectors when Control Areas are re-opened
7. Mapping AVG outbreaks in the field evaluating the potential of eDNA
8. Farm based monitoring protocols

Implications

The workshop provided a forum for key stakeholders to discuss R & D needs following the latest outbreak of AVG and the gaps and analysis of past research carried out by Serge Corbeil.

Three key research areas were identified from the Corbeil research analysis:

- Detection and identification techniques, what the current methods are, what is currently available in Australia and what is futuristic.
- AVG monitoring of both wild and farmed stock for prevalence and infectivity.
- Reducing transmission and infection for wild stock and farmed abalone, possible resistance development such as vaccination or immunity, clarity around disinfection methods and biosecurity practices.

Collection of abalone samples identified under project concept 1 have been collected and are being held in a secure freezer storage in readiness to undertake genotype analysis.

Water samples were collected at the same time as the abalone samples, these are now frozen and being held in secure storage in readiness for eDNA project proposals(s).

Until projects are carried out to understand how the virus spreads, continuing to lock out specific fisheries such as rock lobster will have ongoing implications. Human based transmission vector projects need to be carried out to better understand the virus spread.

VFA have created some information and videos specifically for recreational fishers to help stop the spread of AVG, what diseased abalone looks like and how to wash boats and diving gear post fishing. This information can be found here: https://vfa.vic.gov.au/recreational-fishing/featured/abalone-disease

Recommendations

This project developed several potential research activities as an output from the stakeholder workshop to address key knowledge gaps and biosecurity and monitoring needs.

It is recommended that these project concepts be further developed, and the potential partners engaged with to identify next steps.

ACV received one project proposal titled “Validating patterns of AVG resistance in blacklip abalone” from Adam Miller and Serge Corbeil. The ACDP team had an eight-week window to run challenge tests and getting the project through in a short period of time was not possible.

Samples of abalone and water samples for eDNA research remain in the custody of WADA awaiting further research proposals.

Extension and Adoption

As future projects are developed, extension of these results should continue to be shared with key stakeholders across relevant sectors including abalone wild stock and farmed species, rock lobster, recreational fishers, interstate counterparts, key government agencies and researchers.

Updates and project results should be shared at key meetings or events with the researchers and industry given opportunity to discuss and analyse results.

Appendix 1: Workshop invitees & participants

Key Stakeholders Invited – Workshop 14th December 2021 at Vue Grand Hotel, Queenscliff.

Attended	Name	Position
Y	Craig Fox	**Chairman** Western Abalone Divers Association Abalone Council of Victoria **Director** Abalone Council of Australia Aqua Fox Diving Fox Transport
Y	Harry Peeters	Executive Officer WADA
N	Josh Cahill	Executive Officer Central Zone Abalone Industry Association
Y	Helen Burvill	Executive Officer Eastern Zone Abalone Industry Association
Via Zoom	Dean Lisson	Abalone Council Australia (ACA)
Via Zoom	Stephen Bunney	NSW Abalone Divers Association
Via Zoom	Nicole Hancox	Executive Officer, SA Abalone Divers Association
Y	Mark Gervis	Australian Abalone Growers Association (AAGA)
Y	Tim Rudge	Head of Corporate Development, Yumbah Aquaculture
Via Zoom	Nick Moody	Team Leader, CSIRO-ACDP Fish Diseases Laboratory
Y	Tracey Bradley	Agriculture Victoria
N	Michael Roberts	Ag VIC AVG Phase 2 Response - Project manager
Y	Serge Corbeil	CSIRO
Y	Ian Knuckey	Fishwell Consulting - Facilitator
Y	Chris Padovani	Acting Director Fisheries Management, Science, Policy, Licensing and Communications, VFA
Y	Travis Baulch	Fisheries Manager, VFA
Y	Harry Roeding	Fisheries Manager, VFA
N	John Lloyd	Victoria Rob Lobster Association
N	Ben Scullins	Victorian Recreational Fishing
Via Zoom	Patrick Hone	Director, FRDC
Via Zoom	Chris Izzo	Senior Research Portfolio Manager, FRDC
Via Zoom	Matt Landos	Director, Future Fisheries Veterinary Service
N	Melony Sellars	CEO Genics
Via Zoom	Adam Miller	Deakin University
Via Zoom	Liane Holm	Agribio
Via Zoom	Jeffrey Go	NSW DPI
Viz Zoom	Duncan Worthington	AMBRAD Consulting
N	Peter Kirkland	
N	Paul Hardy Smith	Managing Director, Panaquatic Health Services Pty Ltd

Appendix 2: Workshop agenda

INVITATION ISSUED - 22nd November 2021.
Invitation to attend or participate in an Abalone Viral Ganglioneuritis (AVG) R & D needs workshop.

Abalone Council Victoria (ACV) has been successful in applying to FRDC for and AVG R & D needs analysis and workshop.

BACKGROUND:
Abalone Viral Ganglioneuritis (AVG) disease is caused by a herpesvirus (infection with abalone herpesvirus, AbHV) and was first detected in Victoria in December 2005. The last recorded instance of this disease in Victoria was in January 2010 at Cape Otway. The disease was again confirmed in the Cape Nelson area on 2 May 2021. Since then, further detections have been made at several locations and has been confirmed in Victoria as far east as Cape Otway and as far west as the Discovery Bay Marine Park.

While the virus is active there is a need for the Victorian wild catch abalone fishers to urgently review past research, to inform a gap analysis and further research. This review will support those involved in the outbreak and inform fellow members nationally with a summary of past research and a way forward with other research for up-to-date biosecurity protocols, surveillance, and diagnostic tools to aid rapid detection to future proof the industry. Relevant industry, government representatives and stakeholders will be kept informed and asked to comment and contribute issues and ideas for a cooperative structured way forward to build a common framework on which to establish a set of key actions and or priorities. At the end of a staged process a workshop will be organised to evaluate and reassess research needs, assess and implement research findings and if further gaps are identified develop a framework to action.

OBJECTIVES OF THE PROJECT

1. Conduct an analysis of past research on AVG, create a plain English summary and identify knowledge and research gaps that can be evaluated nationally by key stakeholders
2. Hold a workshop that will be a mixture of face to face and virtual so that key stakeholders can attend and participate in evaluation of suggested research projects identified
3. Prepare and provide a final report that clearly articulates research priorities for AVG in the short term and long term

Serge Corbeil, Experimental Scientist, Fish Disease Lab, Australian Centre for Disease Preparedness (ACDP), CSIRO – commenced at the start of November and has been busy reviewing past research papers to inform the workshop. A plain English summary of the review with an analysis and a list of research papers will be provided to attendees prior to the workshop.

AGENDA

ACV / FRDC AVG R & D NEEDS PLANNING WORKHSOP

VENUE: Vue Grand Hotel, Queenscliff.

DATE: Tuesday 14th December 2021

09.30	Arrival at the Fisheries Office, tea and coffee on arrival	
10.00	Introduction and purpose of the workshop Facilitator	Craig Fox Ian Knuckey
10.30	Overview of findings and gaps	Serge Corbeil
11.30	Questions, agreement on gaps, any additional gaps identified	Group/ Ian Knuckey
12.30	Lunch	
13.30	Group discussion re: R & D issues identified	Group
14.30	Prioritisation exercise	Group
15.30	Top priorities re-visited and confirmed	Ian Knuckey
16.00	Project titles/potential research providers/funding	Ian Knuckey
16.30	Next steps/wrap up	Ian Knuckey/Craig Fox

Zoom conferencing will be made available for those not able to attend in person.

A COVID safe management plan will be provided and is a necessary component of FRDC's funding agreement.

NOTE:

Dr Ian Knuckey from Fishwell Consulting was engaged as the workshop facilitator, Vue Grand Hotel in Queenscliff conference room was the chosen venue and a zoom link was created for those not able to attend in person.

Timing scheduled for Tuesday 14th December 2021 was in between periods of COVID19 venue restrictions and a face-to-face meeting was permitted. However, travelling from interstate was not permitted and virtual zoom link was created for these stakeholders to take part and contribute.

Appendix 3: Presentation from the workshop

Abalone Viral Ganglioneuritis R&D Needs Workshop

Queenscliff 14th December 2021

Workshop objectives

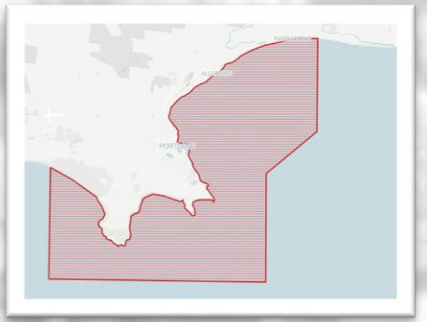
- Overview of past work
  - Findings and gaps
- Evaluation of suggested research projects
- Implementation of research findings
- Prioritisation of future R&D

Workshop Agenda

Time	Item	Presenter
10:00	Introduction and purpose of the workshop	Craig Fox / Ian Knuckey
10:30	Overview of findings and gaps	Serge Corbeil
11:30	Questions, agreement on gaps	Group / IK
12:30	Lunch	
13:30	Group discussion re: R & D issues identified	Group
14:30	Prioritisation exercise	Group / IK
15:30	Top priorities re-visited and confirmed	Ian Knuckey
16:00	Project titles/potential research providers/funding	
16:30	Next steps/wrap up	Craig Fox / Ian Knuckey

R&D Areas

- Detection and identification techniques
  - Current / Available in Aust / Future
  - Specific tissue analyses
- AVG monitoring (wildstock & farmed)
  - Prevalence and infectivity
- Reducing transmission & infection (wildstock & farmed)
  - Resistance development (vaccination, immunity)
  - Disinfection methods
  - Biosecurity practices



Prioritisation

- Why do we care?
- What is our goal(s) here?
- What is the timeframe for achievement?

Immediate work?

- Collections of samples from inside/outside currently infected Victorian populations
  - Samples for research projects
    - DNA collection (tentacle)
      - Genotypes mapping
    - PCR testing
    - Ct levels
    - Other resistance
    - (gene regulation, nutrition pathways, stress-related enzymes)

Immediate sampling work?

- Collections of samples
  - Uninfected populations
    - 3 Sites: Julia Percy / Discovery Bay / Julia Banks / C. Otway
    - 25 randomly collected per site
  - Currently infected populations
    (3 reef codes Yellow Rock, Water Springs, Blowholes)
    - Moribund (50 random per site + water samples)
    - Healthy (50 random per site)
  - Previously infected populations
    (3 sites: Port Fairy, Crags, Water Tower, Leavies)
    - 25 randomly collected per site

  - What are the risks (additional stressors)

Immediate sampling work

- Collections of samples
  - Adam / Serge:
    - Provide collection protocols to WADA
    - Prepare funding application for analysis/reporting
  - WADA / Craig
    - Collect samples during next 14 days as per protocols above
  - VFA
    - Organise transport & storage of samples
    - Look at budget to cover collection of samples with WADA

Medium term work

Preparation for next time….

- Cost-effective and feasible monitoring
  - How do we know when wild stock virus outbreak is coming?
  - How do we know that product from farms is virus-free?
  - How do we know when it is gone?
  - (VFA / AgVIC project)
- Research about human-based transmission vectors
  - Decontamination of vessel-based, equipment-based and animal-based (range of tools, risk-based approach)
  - What minimum management responses are required?
  - Update biosecurity protocols accordingly

Human-based transmission vectors

- Vessels
  - Bilge / ballast / live-holding tanks / anchoring
- Equipment
  - Recreational and various commercial sectors
  - Name and detail with likely importance
    - Bins, dive gear, fishing gear, cray traps,
- Animal-based
  - Farms
  - Wildstock

Human-based transmission vectors

Objectives:

- Undertake a qualitative risk-assessment of human-based AVG transmission vectors (ellicitation process)
  - Vessel / equipment / animal
  - Commercial and recreational
- Based on above, conduct lab trials of each high-risk transmission vector to determine if it supports transmission
- For those proven transmission vectors, trials of intervention methods to inhibit transmission
  - Collaborating with industry, assess the feasible / cost-effective intervention methods
    - UV, fresh water, chemicals, air drying etc etc,
    - Other management protocols
- Report on results to inform future biosecurity protocols

External stressors / risks

- Environmental (physical / biological)
  - Short term
  - Long-term change
- Anthropogenic
- Review of likely stressors that align with previous and current outbreaks

Surveillance of high risk vectors at opening up of closures

- Will be managed by current VFA / AgVIC joinr response project.
- Based on high risk vectors from previous study

Potential of eDNA

- Analyse water samples from current outbreak
  - Need protocols for water sampling and storage
  - How to stop cross-contamination of abalone to water
- Based on results – assess the feasibility of using eDNA to monitor future outbreaks

- Targeted call for analysis methods and costings

Farm-based monitoring protocols

- Use Serge report and VFA / AgVic project as basis to determine virus-free certification protocols
- Compare with current accreditation protocols
- Can we improve the reliability of detection?
  - Testing of the tests
- What is the most cost-effective and efficient option
  - Rapid detection / onsite / cheap
  - Overseas options not currently allowed/accepted in Australia
- Can we get multi-jurisdictional support for these protocols to be accepted in the accreditation procedures

References

Bai C-M, Li Y-N, Chang P-H, Jiang J-Z, Xin L-S, Li C, Wang J-Y, Wang C-M (2019). Susceptibility of two abalone species, Haliotis diversicolor supertexta and Haliotis discus hannai, to Haliotid herpesvirus 1 infection. J Invertebr Pathol 160, 26–32.

Bai C-M, Li Y-N, Chang P-H, Jiang J-Z, Xin L-S, Li C, Wang J-Y, Wang C-M (2020). In situ hybridization revealed wide distribution of Haliotid herpesvirus 1 in infected small abalone, Haliotis diversicolor supertexta. J Invertebr Pathol 173 doi.org/10.1016/j.jip.2020.107356.

Caraguel CGB, Ellard K, Moody NJG, Corbeil S, William LM, Mohr PG, Cummins DM, Hoad J, Slater J, Crane St-J M (2019). Diagnostic test accuracy when screening for Haliotid herpesvirus 1 (AbHV) in apparently healthy populations of Australian abalone Haliotis spp. Dis Aqua Org 136, 199-207. https://doi.org/10.3354/dao03405.

Chen MH, Kuo ST, Renault T, Chang PH (2014). The development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of abalone herpesvirus DNA. J Virol Methods 196,199-203.

Corbeil S, Williams LM, Bergfeld J, Crane M St-J (2012). Abalone herpesvirus stability in sea water and susceptibility to chemical disinfectants. Aquaculture 326–329, 20–26.

Corbeil S, Williams LM, Warner S, Fegan M, Moody NJG, Mohammad I, Ellard K, Caraguel C, Deveney M, Cowley J, Crane MStJ (2014). Aquatic Animal Health Subprogram: Characterisation of abalone herpes-like virus infections in abalone. Final report for FRDC Project No 2009/032 CSIRO Australian Animal Health Laboratory.

Corbeil S, McColl KA, Williams LM, Slater J, Crane M St-J (2017). Innate resistance of New Zealand päua to abalone viral ganglioneuritis. J Invertebr Pathol 146, 31-35.

Corbeil S and Helbig K (2019). Identification of differentially expressed innate immune genes in the New Zealand päua (Haliotis iris) and the Australian hybrid abalone (H. laevigata X H. rubra) upon immersion challenge with the abalone herpesvirus-1 (HaHV-1). Fisheries Research and Development Corporation, Project 2017-117.

Corbeil S (2020). Abalone Viral Ganglioneuritis. Pathogens 9, 720. doi:10.3390/pathogens9090720

Department of Agriculture Fisheries and Forestry (2008). Operational Procedures Manual—Decontamination (Version 1.0), Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN). Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT. AQUAVETPLAN - Operational Procedures Manual - Decontamination - DAWE

Ellard K (2015). Investigations into the Epidemiology of Abalone Viral Ganglioneuritis in Tasmania. VET699 Project, Master of Veterinary Studies (Veterinary Surveillance). Murdoch University, Perth Western Australia, 95pp.

Freidman C, Wight N, Crosson L, VanBlaricom G,Lafferty K (2014). Reduced disease in black abalone following mass mortality: phage therapy and natural selection. Frontiers in Microbiology, 5, 78.

Hick P, Evans O, Looi R, English C, Whittington RJ (2016). Stability of Ostreid herpesvirus-1 (OsHV-1) and assessment of disinfection of seawater and oyster tissues using a bioassay. Aquaculture 450, 412-421.

Jaroenram W, Owens L (2014). Recombinase polymerase amplification combined with a lateral flow dipstick for discriminating between infectious Penaeus stylirostris densovirus and virus-related sequences in shrimp genome. J Virol Methods 208, 144-151.

Kampf G, Lemmen S, Suchomel M (2020). Ct values and infectivity of SARS-Cov-2 on surfaces. The Lancet – Infectious Diseases 21, (6) page e-141. https://doi.org/10.1016/ S1473-3099(20)30883-5

Kasai H, Muto Y, Yoshimizu M (2005). Virucidal effects of ultraviolet, heat treatment and disinfectants against koi herpesvirus (KHV). Fish Pathology, 40(3), 137-138.

Martins C (2018). Effects of diluted chemical disinfectants on abalone followed by the disinfectants neutralisation in seawater. Project Report, Yumbah Aquaculture. Yumbah – Taste the ocean

Magnadottir B (2006). Innate immunity of fish (overview). Fish and Shellfish Immunol 20 (2), 137-151.

Miller A, Toomey M, Holland O, Croft L, Ahrens C, Sherman C, Hoffmann A, Savva N, Lisson D, Clarke A (2021). Population genomic assessment of Australian Blacklip Abalone for abalone viral ganglioneuritis (AVG) resistance. FRDC Project No 2018-057.

Tu P-A, Shiu J-S, Lee S-H, Pang VF, Wang D-C, Wang P-H (2017). Development of a recombinase polymerase amplification lateral flow dipstick (RFA-LFD) for the filed diagnosis of caprine arthritis-encephalitis virus (CAEV) infection. J Virol Meth 243, 98–104