FINAL REPORT

Determining the susceptibility of remnant populations of abalone previously exposed to AVG

Mark St. J. Crane, Serge Corbeil, Lynette M. Williams and Vin Gannon

January 2012

FRDC Project No. 2009/075

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Non-technical Summary

2009/075	Determining the susceptibility of remnant populations of abalone previously exposed to AVG

PRINCIPAL INVESTIGATOR: Dr Mark Crane

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OBJECTIVES:

1. Determine the susceptibility of remnant populations of abalone previously exposed to AVG in Victoria

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED
Results from this research demonstrate that abalone from various sites along the Victorian coast-line that had experienced outbreaks of AVG remain susceptible to infection and disease. The mature abalone that were sourced from these areas are presumed to be survivors from the previous disease outbreaks and the juvenile abalone are presumed to be the progeny of these survivors. Neither groups demonstrated any resistance to re-infection and appeared to be as susceptible as naïve counterparts sourced from a farm that had no history of AVG.

This knowledge will assist industry and regulators manage the wild abalone fishery into the future.

The recent emergence in Australia of abalone viral ganglioneuritis (AVG) caused by abalone herpes-like virus (AbHV) is recognised as a major commercial threat to both the wild capture and the fledgling aquaculture industries, and an environmental threat to wild populations in general. Previous research has been concerned with development of diagnostic tools and a reliable infectivity and disease model. With these tools now developed we are able to address a number of aspects of the biology of this virus. The primary aims of this project were to determine whether abalone sourced from previously affected reefs that had presumably been exposed to the virus but had survived demonstrate any level of resistance to re-infection. These objectives of the project were met successfully.

KEYWORDS: Abalone herpesvirus, AbHV, abalone viral ganglioneuritis, AVG, PCR, resistance

Acknowledgments

Members of the industry organisations in particular the Abalone Divers' Associations and Great Southern Waters Inc. for obtaining and providing abalone that have been used in this project are acknowledged for their cooperation and support. David Forbes, abalone diver, deserves particular mention for his contribution to the project.

Background

On 2 October 2007, the 2nd Abalone Virus Scientific and Management Forum met to discuss and agree on a number of priorities in response to the emergence in Victoria of Abalone Viral Ganglioneuritis caused by Abalone herpesvirus. This forum was attended by representatives of key stakeholders from around Australia. These included DPI Victoria, DPI Tasmania, wild-capture sectors and the aquaculture sector. It was agreed by all that part of the proposed future work program for AVG would include various studies on the epidemiology of the disease, such as:

- National survey of stocks for PCR testing
- Determination of lethal virus titre
- Bio-vectors and abiotic factors
- Sampling of areas where the virus had been to determine the level of immunity, if any, of remaining stock

FRDC Project 2009/032 (Aquatic Animal Health Subprogram: Characterisation of abalone herpes-like virus infections in abalone) is supported by CSIRO, DPI Victoria, PIRSA-SARDI and AAGA. As part of Project 2009/032, remnant abalone from one site previously exposed to abalone herpes virus is to be tested for resistance. While this might provide some insight concerning the potential for re-infection of surviving abalone, the Victorian industry considered this to be inadequate for gaining an understanding about variation in resistance to re-infection among key disease-affected populations. Various environmental factors, genetics and/or level of exposure may play a role in determining what, if any, resistance has been acquired among different abalone populations. This project involved testing of abalone from an additional 4 sites to ensure that generalisations can be drawn to provide practical application of the results in delivering tangible management outcomes. Effective prevention of recurrence is one highly desirable outcome. However, management strategies to prevent recurrence might be unnecessary if all surviving abalone exhibit strong resistance to re-infection.

Need

Despite Abalone herpesvirus remaining undetected among abalone populations for 8 months prior to December 2009, in December 2009 active virus was identified among diseased abalone located at Cape Otway in Victoria. This presented a number of management and bio-security issues as the abalone industry was approaching the initial stages of recommencement of fishing. At the time, the Victorian abalone industry was the only organisation that was actively involved in monitoring the spread of the AVG virus. It was able to show compelling circumstantial evidence of an association between the spread of the AVG virus and human movement patterns.

Not all abalone in virus-exposed areas died and survivors continue to populate reefs in these areas. Critical hypotheses to be tested included whether survival of these abalone was due to:

i) inherent resistance to the virus or
ii) they were not exposed to a lethal dose (viral titre) or
iii) absence of environmental stressor resulted in only subclinical infection
iv) there was no exposure to the infectious agent

Although FRDC Project 2009/032 (Aquatic Animal Health Subprogram: Characterisation of abalone herpes-like virus infection in abalone) proposed an assessment at one site supporting a remnant abalone population, these results would have been inadequate to make decisions about future management and bio-security arrangements. It has been recognised that there is significant variability in the mortalities between different affected locations. Thus it was appropriate to test multiple remnant abalone population sites to determine whether or not there is variation to virus susceptibility/resistance.

It was unknown whether remnant abalone populations that survived the disease outbreak had inherent resistance to the disease or whether, as a matter of chance, they were fortunate not to be exposed to a dose of the virus, when it spread along the coast, sufficient to cause disease.

This project aimed to provide an indication to industry, fishery managers and other stakeholders about the potential for re-infection among remnant abalone populations. In addition, information was sought to support further development of bio-security strategies to avoid re-infection of remnant populations, development of strategic policies to avoid human-mediated spread of the disease to remnant populations, as well as having a bearing on fishery management strategies to allow for the recommencement of fishing.

Objectives

1. Determine the susceptibility of remnant populations of abalone previously exposed to AVG in Victoria

Methods

1. Wild abalone

VADA arranged for the issue of collection permits, organised harvesting for abalone required from all 5 sites, and delivered specimens to AAHL.

Sites harvested:

Experiment 1: 11 November 2010, **Port Campbell Harbour** in 5 metres of water at 38°37'428"S 142°59'139"E

Experiment 2: 31 January 2011, where the eastern face of Moonlight Head abuts Melanesia Beach, about 500m west of White Cliffs (**Lion Headland**): 38 45 252 S 143 17 824 E. In about 6 metres depth, this area had obvious signs of disease impact.

Experiment 3: 3 May 2011, Collection was conducted at **The Craggs**, Port Fairy, 38 23 374S 142 08 620E. There was an abundance of evidence that the virus had affected this location.

Experiment 4: 22 July 2011, Collection was conducted at **Murrells** at Portland, 38 25 020 S 141 31 333E 14 metres deep, 12.1°C. It was an area that received substantial observable mortalities from the virus.

Experiment 5: 12 August 2011, Warrnambool Breakwater, 38 24 209S 142 28 627E, 5 metres, 13.3°C (collected for FRDC Project No. 2009/032 and will be reported as part of that project).

2. Infectivity trials

Method of exposure: Abalone were exposed to virus by bath immersion rather than intramuscular injection. This more natural exposure ensured that the abalone's natural defences would not be circumvented by direct injection.

Challenge dose: To ensure that an appropriate challenge dose was used that would demonstrate the existence, if any, of resistance, three challenge doses were used that were estimated to be equivalent to a 10% lethal dose (LD_{10}), 50% lethal dose (LD_{50}) and 90% lethal dose (LD_{90}). The challenge doses were prepared from infectious water generated as follows: Six 2-year-old farmed abalone (Jade Tiger from Great Southern Waters, Indented Head, Victoria) are inoculated (i.m.) with a standard dose (determined previously: 100 µL each of an AbHV stock containing ~10^4 gene copies) of stock virus and placed in 8 litres of water which is changed daily. On day 4 post-inoculation (p.i.) the water is collected and analysed by qPCR. Previous experiments have shown that at day 4 post-inoculation there should be ~10^6 viral gene copies mL^{-1}. The three challenge doses were prepared based on the Ct value obtained and using a plasmid standard control to estimate the viral titre (Corbeil et al., 2010). This infectious water was then used to make serial dilutions.

It should be noted that Ct values relate to viral gene copies which may overestimate

infectious dose (i.e. a viral gene copy may not always represent an infectious virus particle because, while the viral nucleic acid target for the PCR may be present the virus may not be fully mature). This is one source of variation that can contribute to variation in the mortality rates between experiments.

Experimental animals: Wild abalone (mature and juveniles) were collected and transported to AAHL where they were placed in the bio-secure aquarium and observed for 2-5 days to ensure that they had recovered from any handling/transportation stress. While these abalone are being "rested", farmed abalone (supplied by Great Southern Waters, Indented Head, Victoria) were obtained and used to prepare the challenge virus. For each exposure trial, 10 juvenile and 10 mature abalone from the wild, and 10 juveniles and 10 mature farmed abalone, were exposed to each dose of virus (low, medium and high) and then placed in individual tanks each containing approximately 1.5 to 2.5 litres aerated seawater (Figure 1). Uninfected control animals, both wild and farmed, were included in each trial.

3. Sampling

Following exposure to virus, abalone were monitored on a daily basis for clinical signs. A full change of water was performed daily. When abalone started to demonstrate clinical signs they were euthanased for laboratory examination (histology and PCR analysis). The experimental period was 10–14 days (from previous experience, it is known that infected abalone will die within 14 days of exposure to a lethal dose of Abalone herpesvirus).

Figure 1. Experimental tanks with air-lines housing individual abalone in approximately 1.5 L seawater

Histopathology. Based on reports from natural disease outbreaks and some preliminary experimental infections, it was known that all moribund animals would die within 24 hours of showing clinical signs (e.g., loose attachment to the substrate). Therefore, moribund abalone were immediately sampled for histopathology and PCR analysis. For sampling, abalone were placed on a bed of ice (covered with a paper towel) for

approximately 5 minutes. The abalone were dissected to expose the pleuropedal ganglion and nerve cords (Figure 2). The neural tissue with some surrounding muscle was removed and placed in formalin for a minimum of 24 h. On the following day the formalin-fixed tissues containing the pleuropedal ganglion and nerve cords were prepared by routine histological procedures including dehydration through an alcohol series, paraffin embedding, sectioning (3-6 µm), and staining with haematoxylin and eosin.

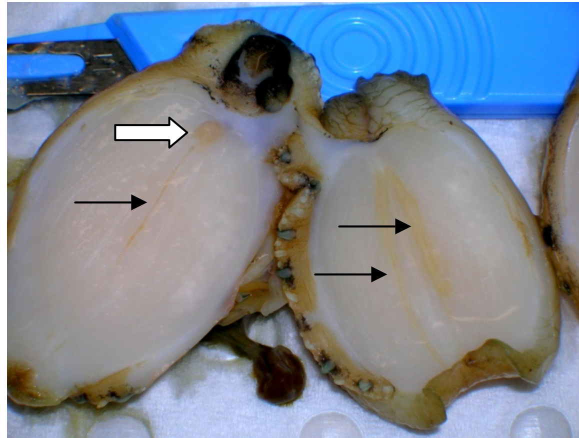

Figure 2. An abalone dissected to reveal the pleuropedal ganglion (white arrow) and nerve cords (black arrows)

4. Real-time PCR

Tissue containing the pleuropedal ganglion was dissected from moribund or dead animals and processed for PCR analysis using a real-time PCR (Corbeil et al., 2010). Nucleic acid was extracted from tissue samples using the QIAamp DNA® mini kit (Cat # 51306) following the manufacturer's instructions. Each sample was tested in duplicate. The Ct cut-off value for a positive result is 35.8, i.e., mean Ct values >35.8 are considered negative and mean Ct values <35.8 are considered positive.

Results and Discussion

1. Experiment 1 (exposure of abalone from Port Campbell Harbour)

Mature (50 animals measuring approx.150 mm shell length) and juvenile (50 measuring 70 mm shell length) abalone were exposed to AbHV in the three experimental groups. Negative (unexposed) control groups were included for both mature and juvenile age classes. Prior to exposure, two wild juvenile and two wild mature abalone were processed for PCR analysis.

Abalone were monitored on a daily basis for signs of disease. Moribund animals were processed for laboratory examination (analysis by histology and PCR). Dead abalone were processed for PCR only (due to autolysis, dead animals are not useful for histological examination). Based on previous experience, moribund animals do not recover and are included in data to generate mortality/survival curves.

Cumulative Mortality

None of the uninfected control abalone died during the course of the experiment. These abalone were sacrificed at the end of the experiment and processed for histology and PCR analysis. The mortality curves for the exposed abalone (both wild and the positive control farmed groups) are shown in Figure 3.

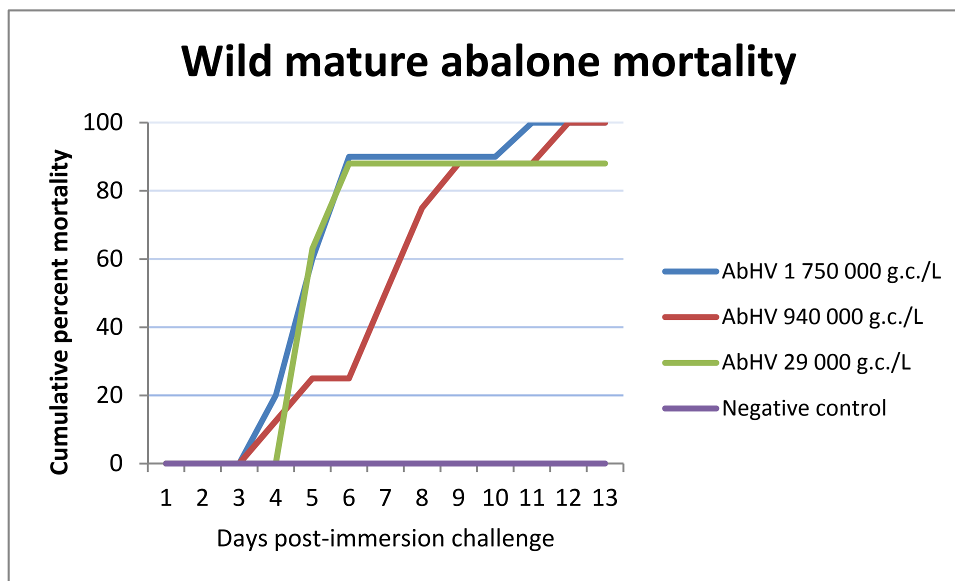

Figure 3a. Mortality curves for mature abalone taken from Port Campbell Harbour and exposed to 3 different doses of Abalone herpesvirus

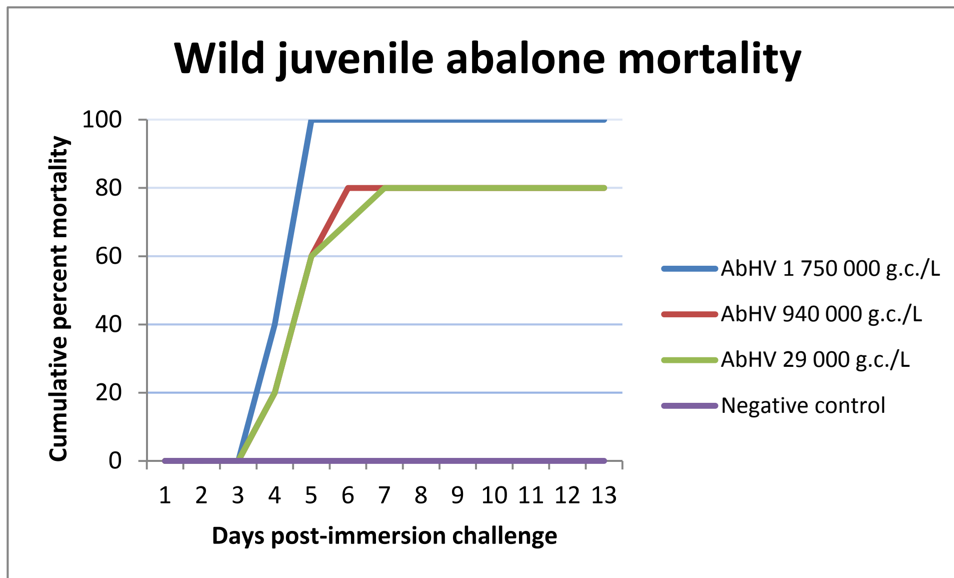

Figure 3b. Mortality curves for juvenile abalone taken from Port Campbell Harbour and exposed to 3 different doses of Abalone herpesvirus

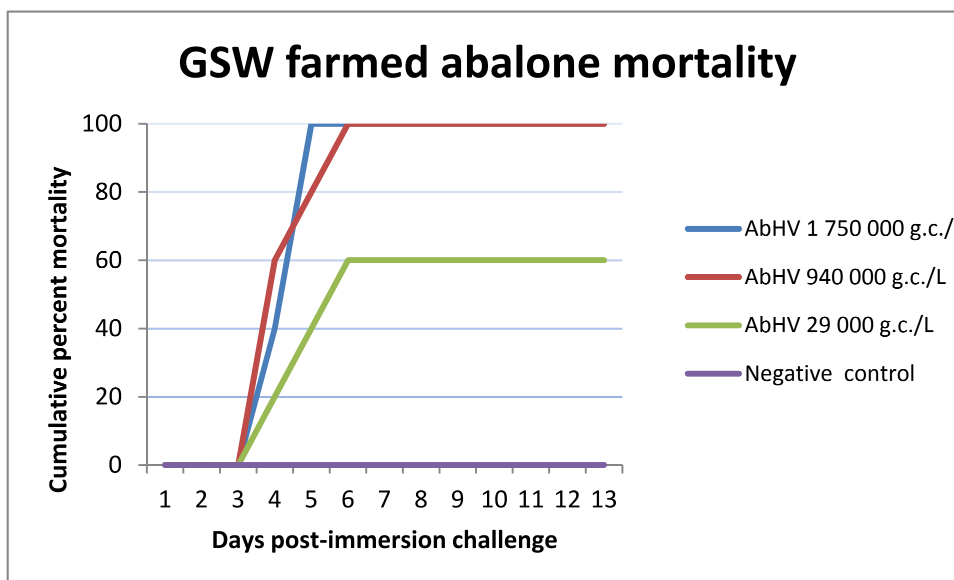

Figure 3c. Mortality curves for farmed abalone supplied by Great Southern Waters Inc. and exposed to 3 different doses of Abalone herpesvirus

PCR Analysis

. Results of the PCR analysis (Table 1) show that all exposed abalone developed infection with those exposed to the high dose developing high levels of infection. Those abalone exposed to the medium and low doses appeared, in general, to show higher Ct values indicating a lower viral load, but still sufficient to caused morbidity/mortality.

Table 1. Summary of PCR Analysis of Abalone exposed to Abalone Herpesvirus (experiment 1)

	Range in mean Ct values			
Experimental group	**High dose**	**Medium dose**	**Low dose**	**Uninf. Co.**
GSW	ND*	11.6–15.9	10.9–34.6	>35.8
Wild mature	11.5–18.9	14.0–35.1	14.3–36.4	>35.8**
Wild juvenile	9.8–11.8	9.2–30.6	9.0–32.6	>35.8

*Not done

**All abalone in this group, except one, had Ct values >35.8. The Ct value for the exception was 34.8 and was therefore a suspect positive. The sample was retested and resulted in a Ct value of 34.6. Whether this result indicates a low level of infection or a false positive is unknown.

Histology

Where possible (in some groups abalone died before samples could be taken for histology) moribund abalone were processed for histology. Representative abalone from each of the groups were examined for lesions. Uninfected control abalone showed no histopathology. Abalone obtained from exposed groups showed lesions typical of abalone viral ganglioneuritis (AVG).

Conclusion

All results, mortality rates, histopathology and PCR analysis, indicate that wild abalone, both mature and juveniles, from Port Campbell Harbour were not resistant to infection and disease.

2. Experiment 2 (exposure of abalone from Lion Headland)

A similar pattern emerged when abalone from Lion Headland were exposed to Abalone herpesvirus.

Cumulative Mortality

All experimental groups of abalone (wild, mature and juvenile; farmed mature and juvenile) exposed to the three doses of abalone herpesvirus showed high mortality rates (Figures 4a-d).

Histology

While uninfected control abalone showed no histopathology (the negative control abalone that died with a low Ct value (see Table 2) could not be processed for histology due to autolysis; exposed abalone demonstrated lesions typical of AVG; abalone exposed to low infectious dose showed mild-to-moderate lesions, while those exposed to the medium and high doses showed moderate-to-severe lesions.

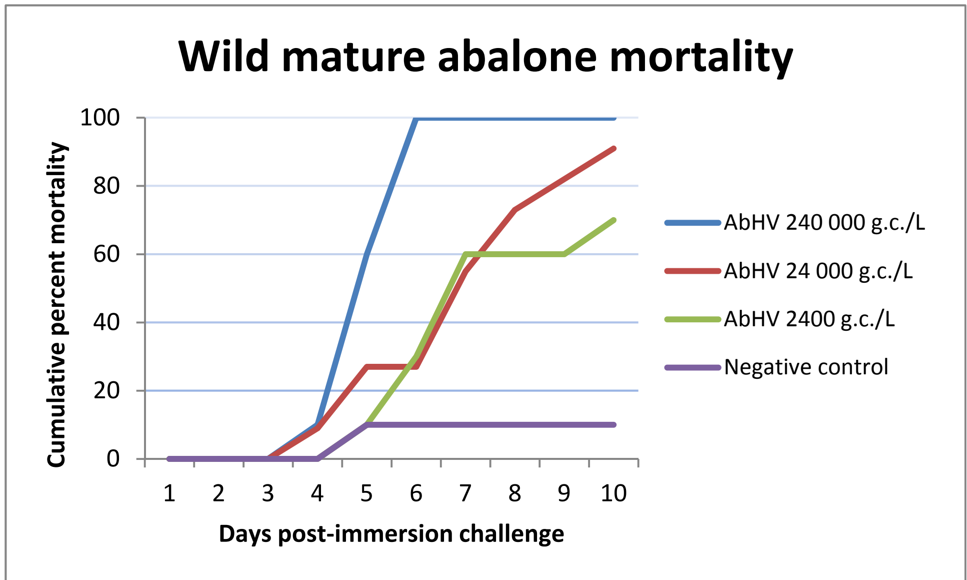

Figure 4a. Mortality curves for mature abalone taken at Lion Headland and exposed to 3 different doses of Abalone herpesvirus

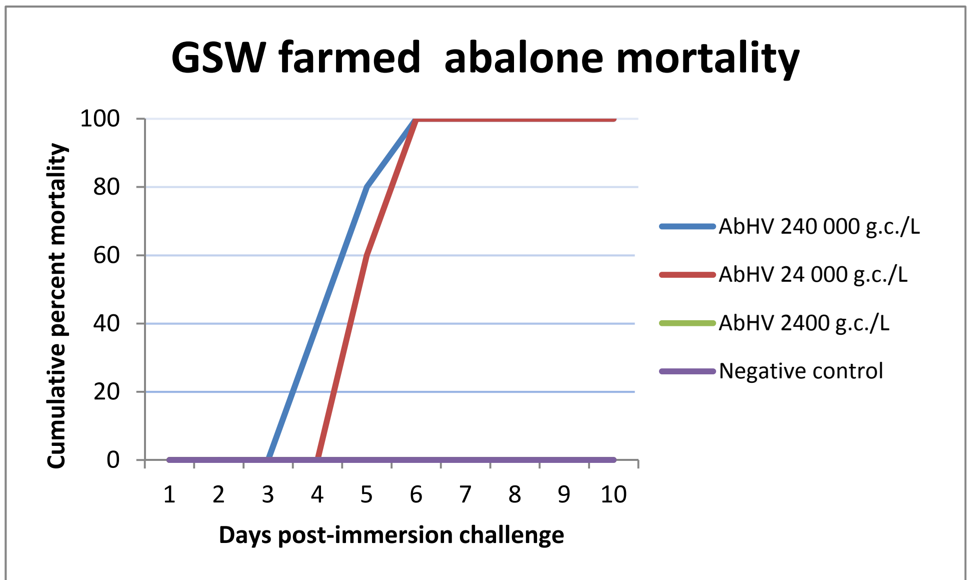

Figure 4b. Mortality curves for mature farmed abalone and exposed to 3 different doses of Abalone herpesvirus

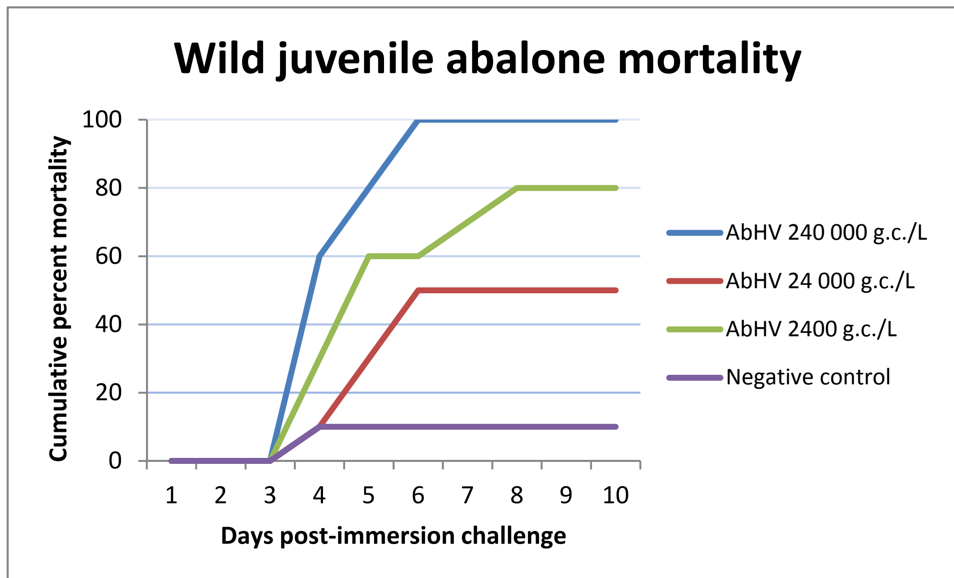

Figure 4c. Mortality curves for juvenile abalone taken at Lion Headland and exposed to 3 different doses of Abalone herpesvirus

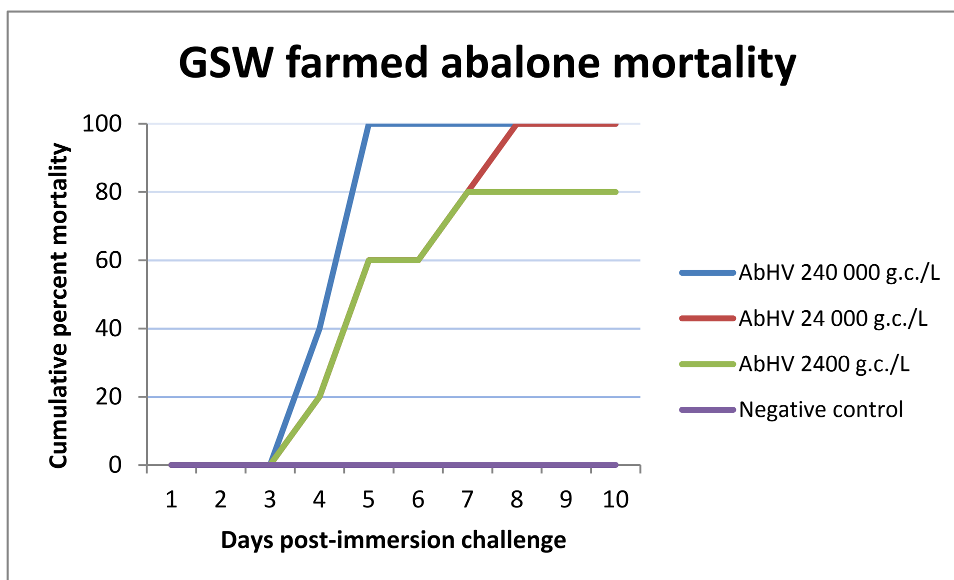

Figure 4d. Mortality curves for juvenile farmed abalone and exposed to 3 different doses of Abalone herpesvirus

PCR Results

PCR analysis (Table 2) demonstrated that the moribund/dead abalone had high levels of infection with AbHV. It is noted that in this experiment only, some of the uninfected negative control abalone demonstrated low Ct values indicating that they were infected. We suspect that there was some cross-contamination of these samples – this is the most likely explanation otherwise more abalone in the negative control groups should have died. This observation does not influence the primary objective of the study – to determine the susceptibility of the wild-sourced abalone.

Table 2. Summary of PCR Analysis of Abalone exposed to Abalone Herpesvirus (experiment 2)

	Range in mean Ct values			
Experimental group	**High dose**	**Medium dose**	**Low dose**	**Uninf. Co.**
GSW juvenile	11.6–17.4	11.0–27.8	13.1–28.0	32.6–39.8
GSW mature	11.9–17.9	14.4–16.5	30.1–35.8	18.4–38.4
Wild mature	9.0–19.1	14.1–24.1	11.2–33.8	32.1–45.0
Wild juvenile	9.5–14.5	9.0–38.6	11.4–37.3	24.3–40.2

Conclusions

Mortality curves, histology and PCR results with exposure of wild abalone from Lion Headland indicate that these abalone were as susceptible to infection and disease as the standard experimental abalone (GSW hybrids).

3. Experiment 3 (exposure of abalone from The Craggs, Port Fairy)

A similar pattern emerged when abalone from The Craggs, Port Fairy were exposed to Abalone herpesvirus.

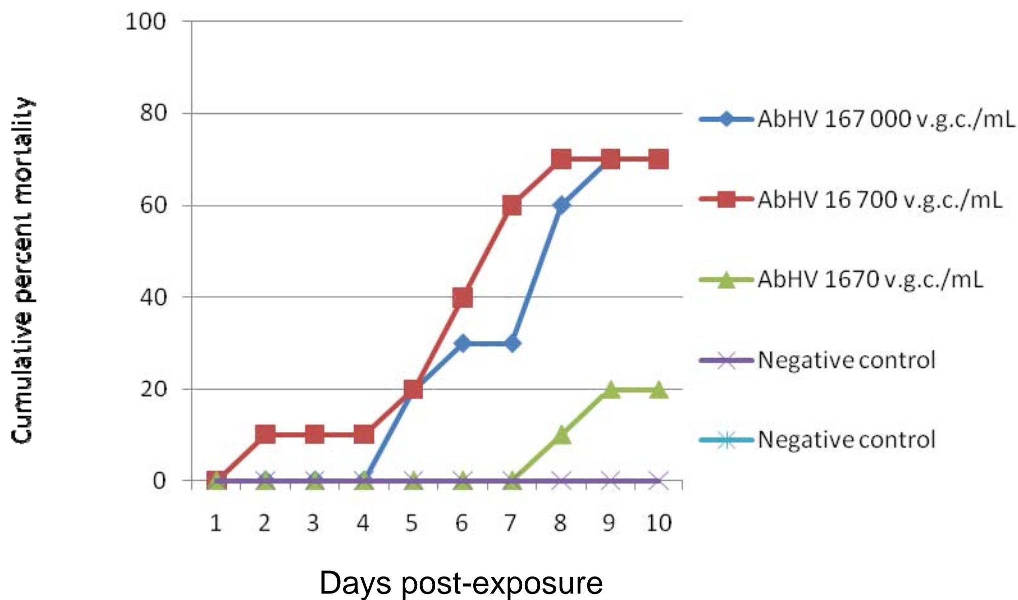

Figure 5a. Mortality curves for mature abalone taken at The Craggs, Port Fairy and exposed to 3 different doses of Abalone herpesvirus

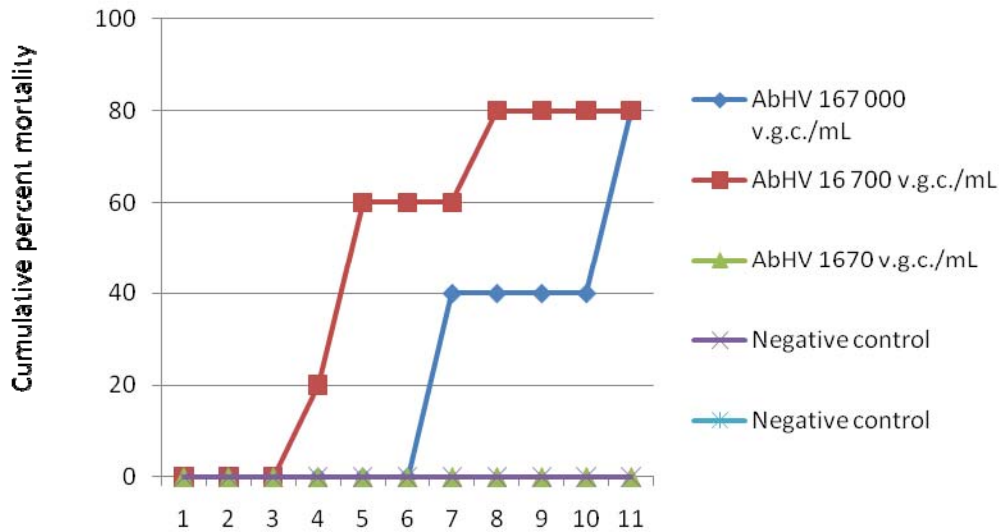

Figure 5b. Mortality curves for mature farmed abalone exposed to 3 different doses of Abalone herpesvirus

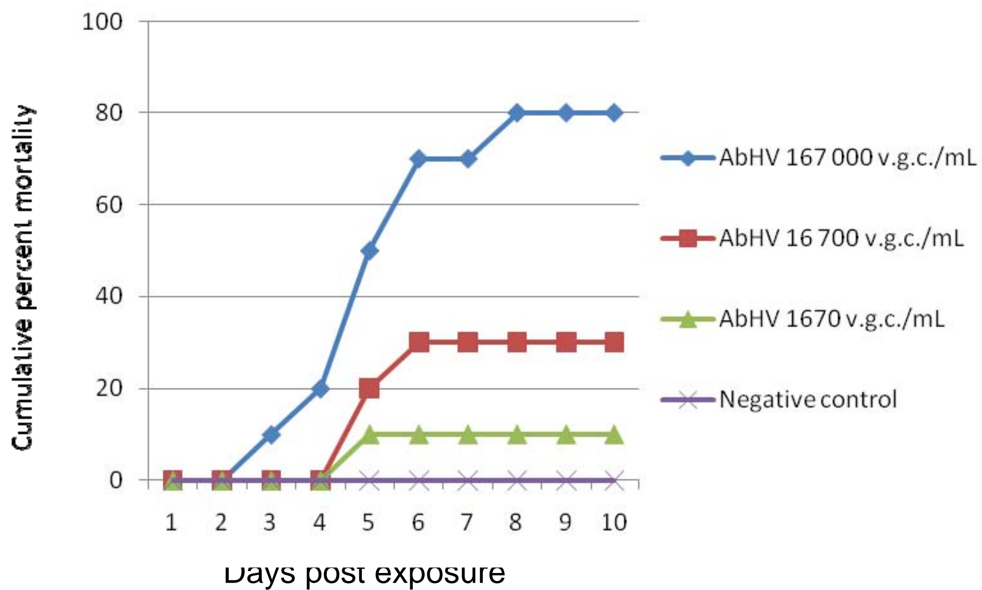

Figure 5c. Mortality curves for juvenile abalone taken at The Craggs, Port Fairy and exposed to 3 different doses of Abalone herpesvirus

Cumulative Mortality

All experimental groups of abalone (wild, mature and juvenile; farmed mature and juvenile) exposed to the three doses of abalone herpesvirus showed high mortality rates (Figures 5a–d).

Histology

While uninfected control abalone showed no histopathology, exposed abalone that were moribund and were processed for histology demonstrated lesions (moderate) typical of AVG.

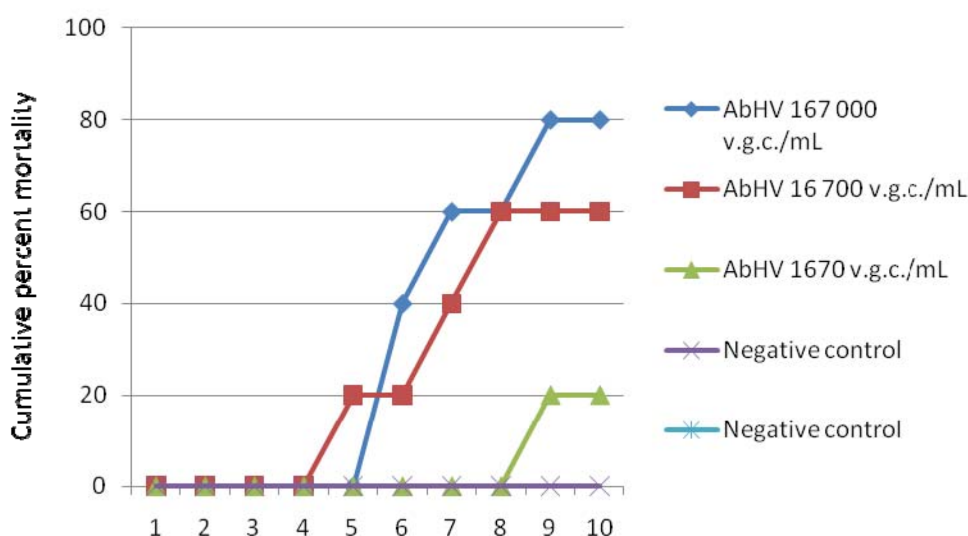

Figure 5d. Mortality curves for juvenile farmed abalone exposed to 3 different doses of Abalone herpesvirus

PCR Results

PCR analysis confirmed that the exposed abalone were infected with Abalone herpesvirus (Table 3).

Table 3. Summary of PCR Analysis of Abalone exposed to Abalone Herpesvirus (experiment 3)

	Range in mean Ct values			
Experimental group	**High dose**	**Medium dose**	**Low dose**	**Uninf. Co.**
GSW juvenile	11.1–29.5	15.0–30.1	31.0–45.0	37.5–45.0
GSW mature	12.9–45.0	15.7–45.0	43.8–45.0	39.0–40.2
Wild mature	12.6–45.0	12.1–41.5	16.5–45.0	37.0–45.0
Wild juvenile	12.3–41.6	12.8–40.5	11.1–42.8	40.0–45.0

Conclusions

Mortality curves, histology and PCR results with exposure of wild abalone from The Craggs Port Fairy indicate that these abalone were as susceptible to infection and disease as the standard experimental abalone (GSW hybrids).

4. Experiment 4 (exposure of abalone from Murrells, Portland)

A similar pattern emerged when abalone from Murrells Portland were exposed to Abalone herpesvirus.

Cumulative Mortality

All experimental groups of abalone (wild, mature and juvenile; farmed mature and juvenile) exposed to the three doses of abalone herpesvirus showed high mortality rates (Figures 6a-d).

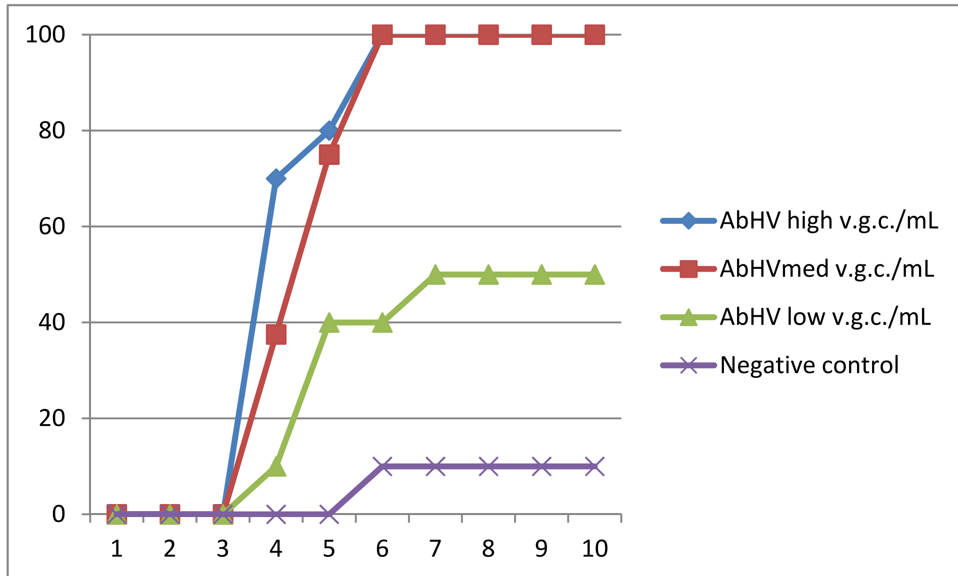

Figure 6a. Mortality curves for juvenile abalone taken at Murrells Portland and exposed to 3 different doses of Abalone herpesvirus

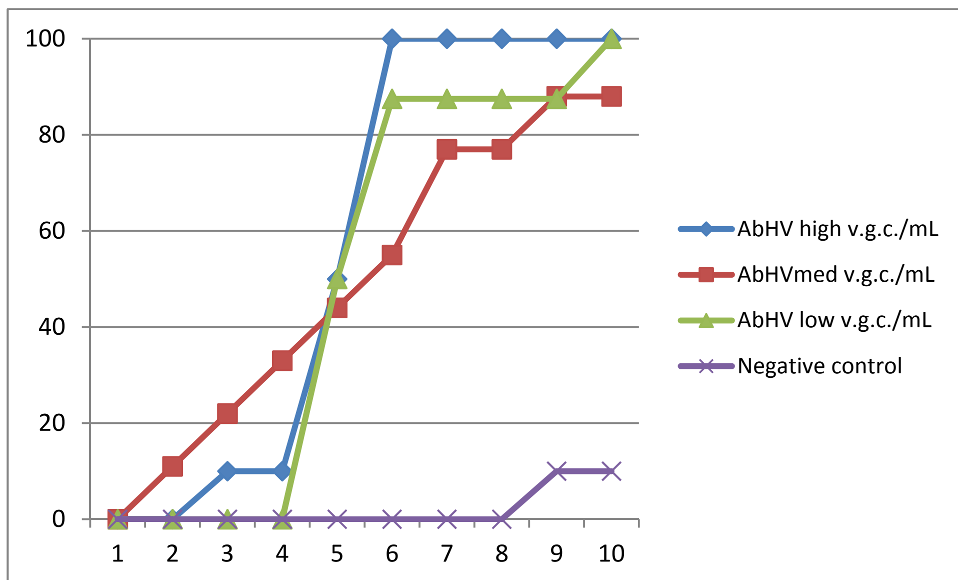

Figure 6b. Mortality curves for mature abalone taken at Murrells Portland and exposed to 3 different doses of Abalone herpesvirus

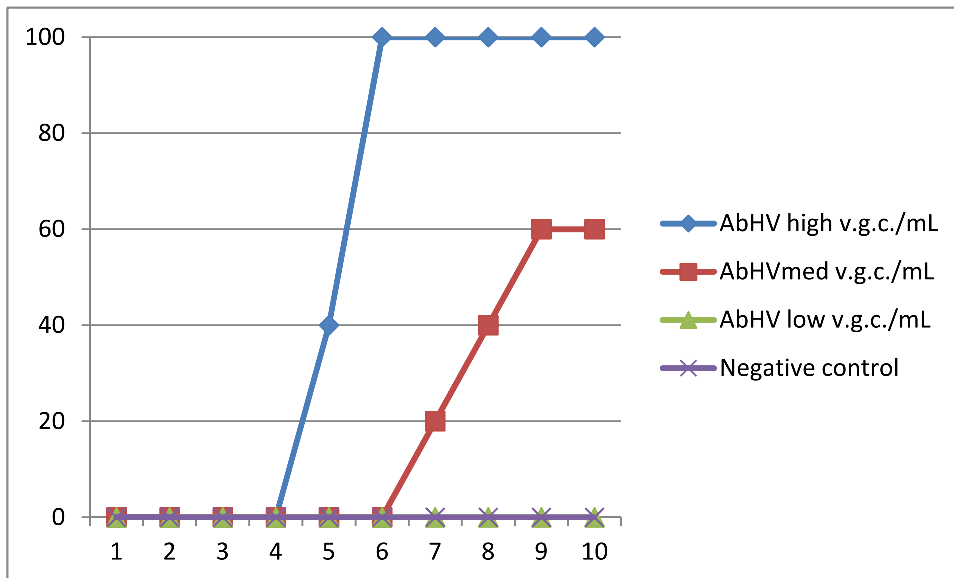

Figure 6c. Mortality curves for juvenile farmed abalone exposed to 3 different doses of Abalone herpesvirus

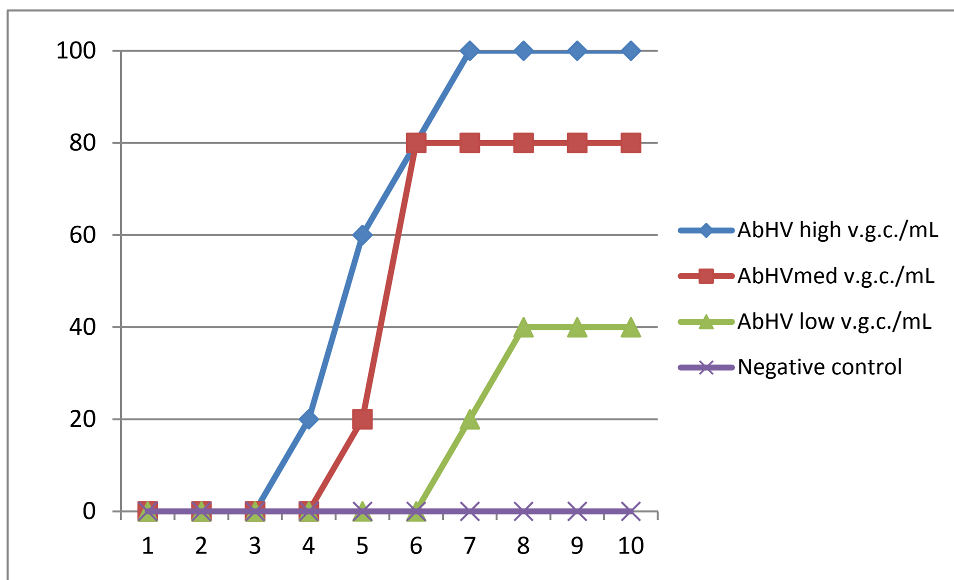

Figure 6d. Mortality curves for mature farmed abalone exposed to 3 different doses of Abalone herpesvirus

Histology

While uninfected control abalone showed no histopathology, exposed abalone demonstrated lesions typical of AVG; abalone exposed to low infectious dose showed mild-to-moderate lesions, while those exposed to the medium and high doses showed moderate-to-severe lesions.

PCR Results

PCR analysis confirmed that the exposed abalone were infected with Abalone herpesvirus (Table 4). As seen with abalone from other sites, there were some negative control samples from wild abalone with positive Ct values (<35.8) which is suggestive but not conclusive of low level infection in the wild.

Table 4. Summary of PCR Analysis of Abalone exposed to Abalone Herpesvirus (experiment 4)

	Range in mean Ct values			
Experimental group	**High dose**	**Medium dose**	**Low dose**	**Uninf. Co.**
GSW juvenile	12.9–20.6	14.6–41.2	39.2–45.0	35.3–45.0
GSW mature	12.2–17.3	13.2–38.3	12.8–45.0	35.4–45.0
Wild mature	11.5–34.7	11.4–45.0	11.1–17.9	26.7–45.0
Wild juvenile	13.1–17.6	13.9–24.5	13.0–45.0	36.9–45.0

Conclusions

Mortality curves, histology and PCR results with exposure of wild abalone from Murrells Portland indicate that these abalone were as susceptible to infection and disease as the standard experimental abalone (GSW hybrids).

Experiment 5 (abalone collected from Warrnambool Breakwater)

This experiment is not reported here but will be reported as part of FRDC Project No. 2009/032: Aquatic Animal Health Subprogram: Development of molecular diagnostic procedures for the detection and identification of herpes-like virus of abalone (*Haliotis* spp.)

Benefits

Industry sectors that will benefit from these outcomes include abalone wild-capture in Tasmania, New South Wales, Victoria, Western Australia and South Australia. Demonstration that abalone present in previously affected areas remain susceptible to infection with AbHV and disease (AVG) will assist industry and State officers to implement a disease management plan.

Further Development

Results from this study suggest that there are wild abalone in Victorian waters with low-level, sub-clinical infections with AbHV. It would be worthwhile confirming that previously exposed survivors carry low level infections. This issue will be partly addressed by FRDC Project No. 2009/032 (Aquatic Animal Health Subprogram: Characterisation of abalone herpes-like virus infections in abalone) in which there is effort going into development of improved diagnostic tests for detection of infection.

All data generated during the course of this project is stored electronically according to CSIRO policies and procedures. While data storage is managed centrally by CSIRO Information Management &Technology, all data generated by specific projects is accessible by the Principal Investigator and the project team members.

Planned Outcomes

Remnant abalone populations have commenced recovery in previously infected sites in western Victoria. It is of great importance to the abalone industry and fishery managers to determine whether or not these surviving populations have developed some resistance to the AVG virus.

The project's outputs have contributed to the planned outcomes as follows:

Results from this project have provided a clear demonstration that abalone inhabiting reefs that have been previously affected by Abalone herpesvirus remain susceptible to infection and disease. This knowledge will allow industry and state regulators manage the abalone fishery accordingly.

Development of further understanding of the biology of abalone herpes-like virus will provide State authorities/industry with an enhanced capability for future management of abalone fisheries. Increased knowledge will lead to reduced impacts of AVG on abalone resources.

The results of this project are publically available:

- The non-technical summary will be included in the FRDC Aquatic Animal Health Subprogram's Newsletter, *Health Highlights*.
- The Executive Officer of VADA is a co-investigator on the project and has provided reports to his constituents.
- Results will be presented at the International Abalone Symposium, Hobart, May 2012.
- The Final Report is publically available from FRDC.

Conclusions

Following the initial outbreak of AVG in wild stock, disease and mortality spread along the Victorian coast-line. However, not all abalone in virus-exposed areas died and currently survivors continue to populate reefs in these areas. It is not clear whether or not the survivors of the disease outbreak either possess some inherent resistance to the virus; they were not exposed to a lethal dose (viral titre); absence of an environmental stressor resulted in only subclinical infection; or, by chance, there was no exposure to the infectious agent.

The objective of this project was to determine the susceptibility of remnant populations of abalone previously exposed to AVG in Victoria with the aim to identify any populations that may possess some level of viral resistance. Thus mature and juvenile abalone sourced from different areas along the Victorian coast-line that had previously been exposed to Abalone herpesvirus, were tested for the level of susceptibility/resistance to infection and disease relative to farmed hybrid abalone in the standard infectivity model. A range in viral titres was used to ensure that any subtle differences in susceptibility/resistance would be detected. Thus the primary objective of the project has been met; results demonstrated clearly that wild abalone sourced from areas previously exposed to the virus were as susceptible to infection as the farmed hybrids and do not demonstrate detectable levels of resistance.

In addition, serendipitously, it was noted that some of the wild abalone, used as negative controls, tested qPCR-positive for the presence of virus with relatively high Ct values (equivalent to presence of low viral titres). These abalone were healthy on harvest and remained healthy during the course of the experiments indicating that sub-clinical infections were present in wild populations. Presumably the state of the host and environmental conditions were such that disease was averted in these animals

It could be argued that this is similar to the situation in Tasmania where evidence suggests that the virus is endemic and present sub-clinically in wild abalone (prevalence currently unknown). Disease outbreaks occur in processing plants presumably due to adverse environmental conditions causing stress in the abalone and increased viral replication to titres sufficient to cause disease. The presence of sub-clinical infection provides an opportunity to study the effect of stressors on expression of disease in attempts to identify the principle stress factors associated with abalone viral ganglioneuritis and, possibly, other potential viral diseases in abalone/molluscs.

References

Corbeil S, Colling A, Williams LM, Wong FYK, Savin K, Warner S, Murdoch B, Cogan NOI, Sawbridge TI, Fegan M, Mohammad I, Sunarto A, Handlinger J, Pyecroft S, Douglas M, Chang PH and Crane MStJ. 2010. Development and validation of a TaqMan PCR assay for the Australian abalone herpes-like virus. *Dis Aquat Org* **92:** 1-10.

Appendix 1 - Intellectual Property

All information arising from this project has been used for the development and/or establishment of standard diagnostic procedures for use by diagnostic laboratories. No intellectual property has been identified.

Appendix 2 – Staff

Name	Position	Organisation
Dr Ken McColl	Senior Research Scientist	AAHL Fish Diseases Laboratory
Dr Serge Corbeil	Experimental Scientist	
Dr Mark Crane	Project Leader, AFDL	
Ms Lynette Williams	Senior Technical Officer	
Dr Jemma Bergfeld	Veterinary Pathologist	Australian Animal Health Laboratory (AAHL)
Vin Gannon	Executive Officer, VADA	VADA
David Forbes	Abalone diver	