FINAL REPORT

# Aquatic Animal Health and Biosecurity Subprogram: 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)

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Aquatic Animal Health and Biosecurity Subprogram: 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)
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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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# Abbreviations

| | |
|---|---|
| AAHL | Australian Animal Health Laboratory |
| AVG | Abalone viral ganglioneuritis |
| CSIRO | Commonwealth Science and Industrial Research Organisation |
| HaHV-1 | Haliotid herpesvirus-1 (Abalone herpesvirus-1) (AbHV-1) |
| IRF-1 | Interferon regulatory factor – 1 |
| mRNA | Messenger ribonucleic acid |
| Poly I:C | Polyinosinic:polycytidylic acid |
| RL-7 | Ribosomal protein-7 |
| RNA | Ribonucleic acid |

# Executive Summary

This project was carried out by scientists from AAHL CSIRO (Dr Serge Corbeil) and from LaTrobe University (Drs Karla Helbig and Subir Sarker). After discovering the existence of an abalone species (Päua - *Haliotis iris*) resistant to AVG our research team based in Geelong and Melbourne undertook (2018) to expose the AVG resistant päua and the AVG susceptible greenlip x blacklip hybrid abalone to HaHV-1 (the etiological agent of AVG) and look for differential gene expression between species. Cutting edge sequencing technology and bio-informatic analysis allowed us to investigate the gene expression of the animals at the molecular level. This approach led to pinpoint abalone genes that are likely to play a role in the protection against AVG in päua. Furthermore, the identification of these genes may facilitate (if applicable) the use of a gene silencing technology such as the CRISPR system in vitro and in vivo to improve immune response to AVG. A breeding program strategy could also eventually be implemented to increase resistance to AVG in susceptible abalone species.

# Background

Haliotid herpesvirus-1 (HaHV-1) (previously known as abalone herpesvirus; AbHV-1) first observed in 2005/6 in land-based abalone farms, spread through wild abalone populations along the coast of the State of Victoria causing the disease known as abalone viral ganglioneuritis (AVG). This resulted in high mortalities in wild abalone and considerable financial losses for the fisheries and abalone farms. All Australian abalone species tested to date have been shown to be susceptible to HaHV-1 infection and AVG (Corbeil et al., 2016). In addition, *Haliotis diversicolor* Reeve 1846 and *H. diversicolor* supertexta cultivated in Asia also suffered significant mortalities due to AVG (Wang et al., 2004; Chang et al., 2005). Since its emergence in Australia, much research on HaHV-1 has been done on its biology and interaction with its host (Corbeil et al., 2012a, 2012b; Crane et al., 2013). In addition, concerns were raised in relation to an eventual introduction of Haliotid herpesvirus-1 into New Zealand waters where the päua (*Haliotis iris*) constitutes an important fishery. To evaluate the susceptibility of the päua to AVG a study was therefore carried out at AAHL and showed for the first time that an abalone species was highly resistant to HaHV-1 infection as well as the disease (Corbeil et al., 2017). However, the underlying mechanism of protection remained unknown. In order to learn more about this protective mechanism(s) we decided to investigate, at the molecular level, the immune response of the animals when challenged with the virus.

# Aims/objectives

The project aims to identify whether differential innate immune gene expression exists between two species of abalone, the AVG resistant päua and the AVG susceptible hybrid abalone after being exposed to the etiological agent HaHV-1.

# Methodology

AVG resistant päua and AVG susceptible hybrid abalone were exposed to HaHV-1 via immersion. At various time points after virus challenge abalone were harvested and total RNA was extracted from different organs. A RNA library was constructed for each species followed by a transcriptome assembly. Subsequently, the transcriptome expression patterns were used to determine differentially expressed genes across treatments.

# Results/key findings

*Immune genes:*

Both the immune genes, IRF-1 and viperin were upregulated in haemocytes of hybrid abalone and päua at 48 hrs post- challenge with HaHV-1 in comparison with the negative control group. In addition, the expression of these immune genes were higher in the päua. No upregulation was visualised at 24 hrs post-challenge. Also, viperin was upregulated in the gills of the päua at 72 hours post-viral challenge.

Additionally, in the haemocytes from abalone stimulated with the RNA viral mimic (poly-I:C), there was a clear distinction between levels of mRNA expression of both IRF-1 and viperin between the hybrid abalone and the päua, with the hybrid species displaying a stronger upregulation 48 hrs post-injection.

Following viral mimic stimulation of the two abalone species, a total of 689 and 361 transcripts were found to be upregulated in the haemocytes of the hybrid and the päua species respectively, with 149 and 70 transcripts downregulated respectively. Interestingly, there was very little overlap between the gene sets regulated between these two species following viral mimic stimulation, indicating that their initial response to a virus is likely to be significantly different.

Many of the upregulated genes within the hybrid abalone following viral mimic stimulation are currently uncharacterised, and as such it is difficult to describe a trend in gene signature for that species. However, in respect to the päua, many of the early upregulated genes belong to extracellular matrix protein families, including multiple sub-species of collagen and matrillin.

*Transcriptional profile of päua versus hybrid abalone to HaHV-1:*

At 48 hours following HaHV-1 challenge of the abalone species, only 6 out of 493 up-regulated transcripts and only 1 out of 185 down-regulated transcripts were common in haemocytes between the two species. Analysis of the gills at 72 hours following challenge of HaHV-1 in both abalone species displayed a similar trend as the haemocytes, with minimal overlap of either the upregulated or downregulated transcripts.

As seen with viral mimic stimulation, there was significant upregulation of transcripts known to be involved in remodelling the extracellular matrix in the päua haemocytes (48 hrs post HaHV-1 challenge); this was not seen in the haemocytes of the hybrid abalone transcriptional response to the virus. However, the hybrid abalone did show evidence of upregulation of genes involved in remodelling the extracellular matrix in the gills at 72 hours, which was not seen in the päua, perhaps demonstrating either a lag in response time of the hybrid abalone to HaHV-1, or a tissue differential expression.

# Implications for relevant stakeholders

Industry: In the eventuality that specific gene(s) are identified as likely candidates involved in resistance against AVG, breeding programs would benefit taking into consideration the importance of the genes as markers to be targeted. Accordingly, associated regulatory DNA sequences (e.g. promoters, repressors etc.) should be identified during genome sequencing of wild abalone (Adam Miller FRDC project # 2018-057) and compared with the päua genome sequences.

# Keywords

Haliotid herpesvirus-1 (HaHV-1), abalone viral ganglioneuritis (AVG), päua, *Haliotis iris*, hybrid abalone (*H. laevigata* x *H. rubra*), gene expression, disease resistance, mRNA sequencing, genomic analysis.

# Introduction

AVG has occurred in Victorian abalone for several years beginning from 2005/6. Later on several genetic variants of the causative agent, haliotid herpesvirus (HaHV-1) (previously called abalone herpesvirus AbHV-1) were discovered in Tasmanian processing plants, however, no disease outbreak was observed in the wild abalone population. All Australian abalone species tested to date have been shown to be susceptible to HaHV-1 infection and to the disease it causes (Corbeil et al., 2016). In addition, a recent study conducted at AAHL revealed that the New Zealand päua (*H. iris*) is highly resistant to AVG when challenged with the virus via intra-muscular injection and immersion (Corbeil et al., 2017). This led us to develop a research project in collaboration with an expert in innate immunity (Dr Karla Helbig, La Trobe University) to attempt to identify immune genes possibly involved in the resistance. Identifying the mechanisms of protection would provide a knowledge-base that could lead to the development of hybrid abalone possessing resistance traits and/or to the development of immunotherapeutic molecules that could protect Australian abalone species. This project aims to address this knowledge gap and is relevant to all jurisdictions with abalone fisheries.

# Objectives

1- Define the time-line of an anti-viral response in the päua and Australian hybrid abalone for the first time, utilising real-time PCR, and a set of known anti-viral effector genes.

2- Through mRNA sequencing and genomic analysis, identify early genes expressed in päua and Australian hybrid abalone upon HaHV-1 immersion challenge.

3- Establish an immune signature in the early response of the host to the virus that differs between the päua and Australian hybrid abalone, to determine key immune players in HaHV-1 resistance.

# Methods

This project sought to first examine a timeline pattern of potential anti-viral gene expression following viral infection of abalone for the first time, to inform potential time points for Next Generation Sequence (NGS) analysis. Transcriptional profiling by NGS was performed initially by utilising viral mimic treatment, (polyI:C (dsRNA)) of both abalone species to assess the anti-viral transcriptome response of abalone haemocytes without the opportunity for immune system interference by viral antagonists. The second set of transcriptional profiling assessed the immune response of both abalone species to HaHV-1 in two tissue types; haemocytes at 48 hours and gills at 72 hours post-challenge to assess potential differences at the tissue level between the two abalone species in response to HaHV-1.

**Challenge trial**

Päua (*H. iris*) and blacklip (*H. rubra*) x greenlip (*H. laevigata*) abalone hybrids were used as experimental animals and trials were conducted in aquaria in the secure bio-containment facility at AAHL CSIRO. The infectious water containing Haliotid herpesvirus-1 (HaHV-1 Vic isolate) (used for immersion challenge experiments) was produced by injecting, intramuscularly in the foot, a set of thirty naïve hybrid abalone with 100 µL of the virus stock solution. Inoculated animals were held in one tank containing 20 L aerated sea water. After 4 days infectious water was harvested to provide the immersion challenge dose. Additionally, both species of abalone were also injected intramuscularly with 50µl of a dsRNA viral analogue (total of 0.25µg poly I:C), to determine the early innate immune response profile of both species in the absence of viral antagonism. Challenged abalone were kept individually in 2L size tanks.

**Samples Harvesting**

At time points 24, 48, 72 and 96 hours after immersion challenge (4 päua/hybrid abalone per time point) haemocytes, gills and nerve tissues were sampled for total RNA extraction, qPCR testing and analysis of viral load. Control infected abalone were also utilised, and samples were stabilised in RNA later and frozen at -20$^{o}$C until tested.

**Total RNA extraction**

A Qiagen RNEasy Mini kit (cat # 74104) was used to extract total RNA from all samples according to the manufacturer's instruction (approximately 20mg of tissue was utilised where possible). The extracted RNA was eluted in 60µl of RNA later solution. RNA was quantified using a NanoDrop 1000 version 3.8.1 (Thermoscientific) and stored at -20$^{o}$C until used. Not all samples after processing were of sufficient quality and quantity to obtain genomic information.

**cDNA synthesis and gene amplification using the SYBR Green assay**

The SuperScript™ III First Strand SYBR Green One-Step qRT-PCR Kit (Life Technologies, cat number:18080400) was used to synthesize cDNAs from extracted total RNA following the manufacturer's instruction. The expression of the house keeping-gene, ribosomal protein 7 (RL-7), as well as the well-characterised inducible anti-viral genes, interferon regulatory factor 1 (IRF-1) and virus inhibitory protein (viperin) was measured using the following primers: RL-7 Fwd 5′-CCAAAYGGRGGCTGGAGAA-3'Rev 5′- CTCCTYAGCAGAGCATTGAT -3',IRF-1 Fwd 5′-GTCGRCWSAAACAGGACAA-3' Rev 5'-GGCCCACAGTTCAAARATACA-3',Viperin Fwd 5′-ATGMGGTTCCTGGATTGTC-3' Rev 5′-TCTCGTCAAAYCCACTGAAGTC-3'.

The assay was carried out in a 25 µl reaction mixture containing: 12.5µl of 2x SYBR Green Reaction Mix, 2.5 uL of each primer (18uM) and 5.5 µl of nuclease-free water plus 2uL of cDNA.

The optimized thermal cycling conditions were as follows:
2 min at 50 °C then 10 min at 95 °C followed by 40 cycles at 95 °C for 15 sec, 55 °C for 60 sec, and 72 °C for 60 sec, followed by a melting curve analysis of 95 °C for 15 sec, 60 °C for 60 sec, 95 °C for 30 sec and 60 °C for 15 sec. Finally, an ABI Prism®□7700 Sequence Detection System and software Sequence Detector version 1.9 (PE Applied Biosystems) was used for the analysis and storage of data.

**Next Generation RNA-seq library preparation**

RNA extraction from abalone tissues often results in both low RNA quality, and low RNA yield. RNA was assessed using a NanoDrop spectrophotometer, and an Agilent 2100 Bioanalyzer (Agilent Technologies, USA), and in conjunction with the results from the initial gene amplification experiments described above, experimental sets of samples were chosen for sequence library preparation utilising the Illumina TruSeq RNA sample preparation v2 kit (Table 1). Note that the samples belonging to the haemocyte 48 hrs control group could not be used due to low RNA quality. Therefore, throughout all analyses we used the samples from the haemocyte 24 hrs control group as comparative reference values.

The protocol for RNA-seq library preparation was adapted from the Illumina TruSeq® RNA sample preparation v2 Kit. Twelve strand-specific Illumina® RNA-seq libraries were generated (three libraries, for each group of control; three replicates for dsRNA at the 24 h stimulation; and six replicates for each group of dsRNA and dsDNA at the 48 hrs stimulation) using 0.5 µg of total RNA. Total RNA was heated at 65°C for 5 min to denature any secondary structure and facilitate binding of the poly(A) RNA to the oligo-dT beads. Purification of poly(A) RNA was performed using Illumina TruSeq® RNA sample preparation v2 Kit according to the manufacturer's instructions (Illumina® Inc., San Diego, CA, USA). Purified poly(A) RNA was then fragmented and primed using 19.5 µL of Elute, Prime and Fragment Mix containing random hexamers (Illumina® Inc., San Diego, CA, USA) for 8 min at 94°C.

Synthesis of first-strand cDNA was performed immediately by incubating fragmented and primed mRNA with first strand master mix (Illumina, USA) and SuperScript® II reverse transcriptase (Invitrogen™) at a ratio of 9:1. The reaction mixtures were run in a thermal cycler at 25°C for 10 min, at 42°C for 50 min, and 70°C for 15 min. Second-strand cDNA synthesis was initiated immediately, by adding 25 µL of second-strand master mix (Illumina® Inc., San Diego, CA, USA) to each well, when the thermal cycle reached 4°C. After gentle and thorough mixing, the plate was incubated in a pre-heated thermal cycle at 16°C for 1 hr. The double-stranded cDNA (ds cDNA) was subsequently purified by using AMPure XP beads (Invitrogen™, USA) according to the manufacturer's instructions and eluted in 50 µL of the resuspension buffer (Illumina® Inc., San Diego, CA, USA).

Blunt-end repair of ds cDNA was performed in a 100 µL reaction containing 10 µL diluted End Repair Control to 1/100 in resuspension buffer and 40 µL of End Repair Mix to each well (Illumina, USA). Reactions were incubated on a pre-heated thermal cycler at 30°C for 30 min and the ds cDNA was cleaned up using AMPure XP beads (Invitrogen™, USA) according to the protocol described in Illumina TruSeq® RNA sample preparation v2 Kit and eluted in 15 µL of the resuspension buffer (Illumina® Inc., San Diego, CA, USA).

To facilitate Illumina® adaptor ligation, a single "A" nucleotide was added to the 3′ ends of the blunt-end-repaired cDNA samples. Fifteen microliters of purified phosphorylated blunt-end-repaired cDNA was included in a final 30 µL reaction mixture containing 2.5 µL diluted A-tailing control to 1/100 in resuspension buffer and 12.5 µL of A-tailing mix to each well (Illumina® Inc., San Diego, CA, USA). The reaction mixtures were run in a thermal cycler at 37°C for 30 min followed by 70°C for 5 min.

Illumina® RNA-seq adaptor ligations were performed in a reaction volume of 37.5 µL containing 30 µL of phosphorylated blunt-ended cDNA in addition to 2.5 µL diluted ligation control, 2.5 µL ligation mix, and 2.5 of custom indexed adaptors to each well (see Table S1 in Supplementary Material for barcode index sequences). Reaction mixtures were incubated for 30°C for 10 min followed by inactivation of the ligation with 5 µL of stop ligation buffer into each well (Illumina® Inc., San Diego, CA, USA). Adaptor-ligated cDNA was purified using AMPure XP beads (Invitrogen™, USA) according to the protocol described in Illumina TruSeq® RNA sample preparation v2 Kit and eluted in resuspension buffer in a final volume of 20 µL.

PCR amplification of selectively enriched DNA fragments (50 µL) was performed using 20 µL of adaptor-ligated cDNA, 5 µL of Illumina® PCR primer cocktail and 25 µL of Illumina® PCR master mix to each well (Illumina® Inc., San Diego, CA, USA). PCR amplification reactions were performed with the following temperature cycling profile: 98°C initial denaturation for 30 s; 15 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 30 s; and 72°C final extension step for 5 min. PCR products were purified to remove PCR-

**Table 1: Treatment samples assessed for transcriptome analysis.**

| ID | species | Treatment | Time_post_challenge (Hour) | Tissue | Rep |
|---|---|---|---|---|---|
| 1_111 | Päua | HaHV-1 | 72 | Gills | 1 |
| 2_113 | Päua | HaHV-1 | 72 | Gills | 2 |
| 3_115 | Päua | HaHV-1 | 72 | Gills | 3 |
| 5_107 | Päua | Control | 72 | Gills | 2 |
| 6_109 | Päua | Control | 72 | Gills | 3 |
| 7_127 | Hybrid | HaHV-1 | 72 | Gills | 1 |
| 8_129 | Hybrid | HaHV-1 | 72 | Gills | 2 |
| 9_133 | Hybrid | HaHV-1 | 72 | Gills | 3 |
| 10_119 | Hybrid | Control | 72 | Gills | 1 |
| 11_121 | Hybrid | Control | 72 | Gills | 2 |
| 12_123 | Hybrid | Control | 72 | Gills | 3 |
| 13_91 | Hybrid | HaHV-1 | 48 | Haemocytes | 1 |
| 14_92 | Hybrid | HaHV-1 | 48 | Haemocytes | 2 |
| 15_94 | Hybrid | HaHV-1 | 48 | Haemocytes | 3 |
| 16_65 | Hybrid | Control | 24 | Haemocytes | 1 |
| 17_67 | Hybrid | Control | 24 | Haemocytes | 2 |
| 18_68 | Hybrid | Control | 24 | Haemocytes | 3 |
| 19_87 | Päua | HaHV-1 | 48 | Haemocytes | 1 |
| 20_88 | Päua | HaHV-1 | 48 | Haemocytes | 2 |
| 21_89 | Päua | HaHV-1 | 48 | Haemocytes | 3 |
| 22_70 | Päua | Control | 24 | Haemocytes | 1 |
| 23_71 | Päua | Control | 24 | Haemocytes | 2 |
| 24_72 | Päua | Control | 24 | Haemocytes | 3 |
| 25_81 | Päua | Poly_I_C | 48 | Haemocytes | 1 |
| 26_82 | Päua | Poly_I_C | 48 | Haemocytes | 2 |
| 27_83 | Päua | Poly_I_C | 48 | Haemocytes | 3 |
| 28_84 | Hybrid | Poly_I_C | 48 | Haemocytes | 1 |
| 29_85 | Hybrid | Poly_I_C | 48 | Haemocytes | 2 |
| 30_86 | Hybrid | Poly_I_C | 48 | Haemocytes | 3 |
| 4_103 | Päua | Control | 72 | Gills | 1 |

generated adaptor-dimers using AMPure XP beads (Invitrogen™, USA) according to the protocol described in Illumina TruSeq® RNA sample preparation v2 Kit with final elution in 30 µL of resuspension buffer.

All RNA-seq libraries were quantified and assessed using an Agilent Tape Station (Agilent Technologies) by the Australian Genomic Research Facility (AGRF, Melbourne) and confirmed insert sizes of 100–125 bp for all individual libraries. Individual RNA-seq libraries were standardized and pooled in equimolar quantities. The quantity and quality of the final pooled library was assessed as described above prior to sequencing by the facility. Cluster generation and sequencing of the pooled RNA-seq libraries were sequenced as paired-end using Illumina® HiSeq HT chemistry according to the manufacturer's instructions.

**Transcriptome Analysis**

Raw fastq files were trimmed to increase the quality of reads (Quality limit = 0.05) as well as mapping performance. Reads with ambiguous nucleotides were also removed from data (ambiguous limit = 2). To increase mapping performance, 25 nucleotides were removed from 5' terminal of all reads due to sequence reading fluctuations. Trimming and quality analysis were performed using CLC Genomics Workbench version 12 (QIGEN).

Recently published draft genome of *Haliotis rufescens* (Red Abalone) (Masonbrink et al., 2019) was used as reference for mapping and expression analysis. To this end, genome assembly and annotation were downloaded from AbaloneDB (https://abalone.dbgenome.org/downloads) of Iowa State University. The assembled draft genome has high quality with 8,371 scaffolds, total length of 1.498 Gb, N50 of 1.895 Mb, and 57,785 annotated genes (Masonbrink et al., 2019).

The following parameters were used for mapping: Mismatch cost (penalty) = 1, Insertion cost = 2, Deletion cost = 2, Length fraction = 0.6, and Similarity fraction = 0.6. Mapping was performed using CLC Genomics Workbench and expression was measured as total gene reads, unique gene reads and RPKM. Total gene reads were used for statistical analysis.

Differential expression analysis was performed based on Generalized Linear Model (GLM), similar to edger package, (Robinson et al., 2010). The p-values were corrected with FDR statistics. The use of the GLM allows us to fit curves to expression values without assuming that the error on the values is normally distributed. Here, we assume that the read counts follow a Negative Binomial distribution. Fold changes which are calculated from the GLM, which corrects for differences in library size between the samples.

# Results and Discussion

**OBJECTIVE 1:**

**Define the time-line of an anti-viral response in the päua and Australian hybrid abalone for the first time, utilising real-time PCR, and a set of known anti-viral effector genes.**
Abalone Viral Ganglioneuritis (AVG) resistant päua and AVG susceptible greenlip x blacklip hybrid abalone were successfully challenged with both the Haliotid herpesvirus (HaHV-1), as well as with a RNA viral mimic (poly I:C). Animals were harvested at three different time points after immersion challenge. Hemolymph, gills and nerve/muscles were harvested from each animal and total RNA was extracted from all samples, followed by cDNA synthesis from each sample.

Real-time PCR has not previously been performed on abalone RNA samples, and as such new protocols needed to be developed. Prior to running the real-time sampling of immune genes on the experimental abalone, we first needed to establish a set of primers that would be useable in assays to test the level of a variety of selected genes, in conjunction with a selected baseline control. We sourced full abalone genomes for both the päua and the hybrid abalone, however, conservation of selected target genes between both abalone species was only found for 2 control genes, RS18, and RL7, as well as the immune genes, IRF-1 and viperin. Redundant primer sequences were generated to ensure that the mRNA from these genes would be targeted in both abalone species, and the primer sets were tested for their ability to specifically detect a single product using real-time syber green based PCR. Subsequent melt-curve analysis revealed that we could utilise RL-7 as a base line control gene for multiple species of abalone, and that detection of both viperin and IRF-1 in the abalone was also possible using this method.

Real-time PCR was next performed on all samples to assess the strength of an anti-viral response in a time course related manner, and in varying tissues. The only tissues displaying acceptable RNA quality and quantity were those taken from haemocytes and gills, and as such, were the only tissues analysed by real-time PCR. As can be seen below (Fig 1), both the immune genes, IRF-1 and viperin were upregulated in haemocytes at 48 hrs post challenge with HaHV-1, with no upregulation visualised at 24 hrs post challenge. Due to RNA quality/quantity, we could only assess levels of these two immune genes at 72 hours post HaHV-1 challenge, with viperin being upregulated in the päua only at 72 hours. Additionally, in the haemocytes from abalone stimulated with the RNA viral mimic, there was a clear distinction between levels of mRNA expression of both IRF-1 and viperin between the two animals, with the hybrid species displaying a stronger upregulation at 48 hrs post-injection.

Due to the small amounts of RNA from these animals, real-time PCR experiments were not performed multiple times, and as such, statistics were not performed. This set of analyses was to assess the correct time point, and potential tissues to carry forward into whole transcriptome profiling of these animals. Following the data analysis, and RNA quality/quantity requirements, the following groupings and comparisons were chosen for further analysis (Table 2).

**Table 2: Whole Transcriptome analysis groupings**

| | Sample comparison (sample Ids) | Sample set (IDs) | What question will this answer? |
|---|---|---|---|
| 1 | Haemocytes Päua + poly I:C, 48hrs (81-83) vs<br>Haemocytes Hybrid + poly I:C, 48hrs (84-86) | 70-72<br>65, 67,68 | This will tell us the normal anti-viral response of each animal, and if it differs between species |
| 2 | Haemocytes Päua + HaHV-1, 48hrs (87-89) vs<br>Haemocytes Hybrid + HaHV-1, 48hrs (91, 92,94) | 70-72<br>65, 67,68 | Is there a difference at the haemocyte gene expression level between HaHV-1 infection of each animal at this time point |
| 3 | Gills Päua + HaHV-1, 72hrs (111,113,115) vs<br>Gills Hybrid + HaHV-1, 72hrs (127,129,133) | 103,107,109<br>119,121,123 | Is there a difference at the gill gene expression level between HaHV-1 infection of each animal at this time point |

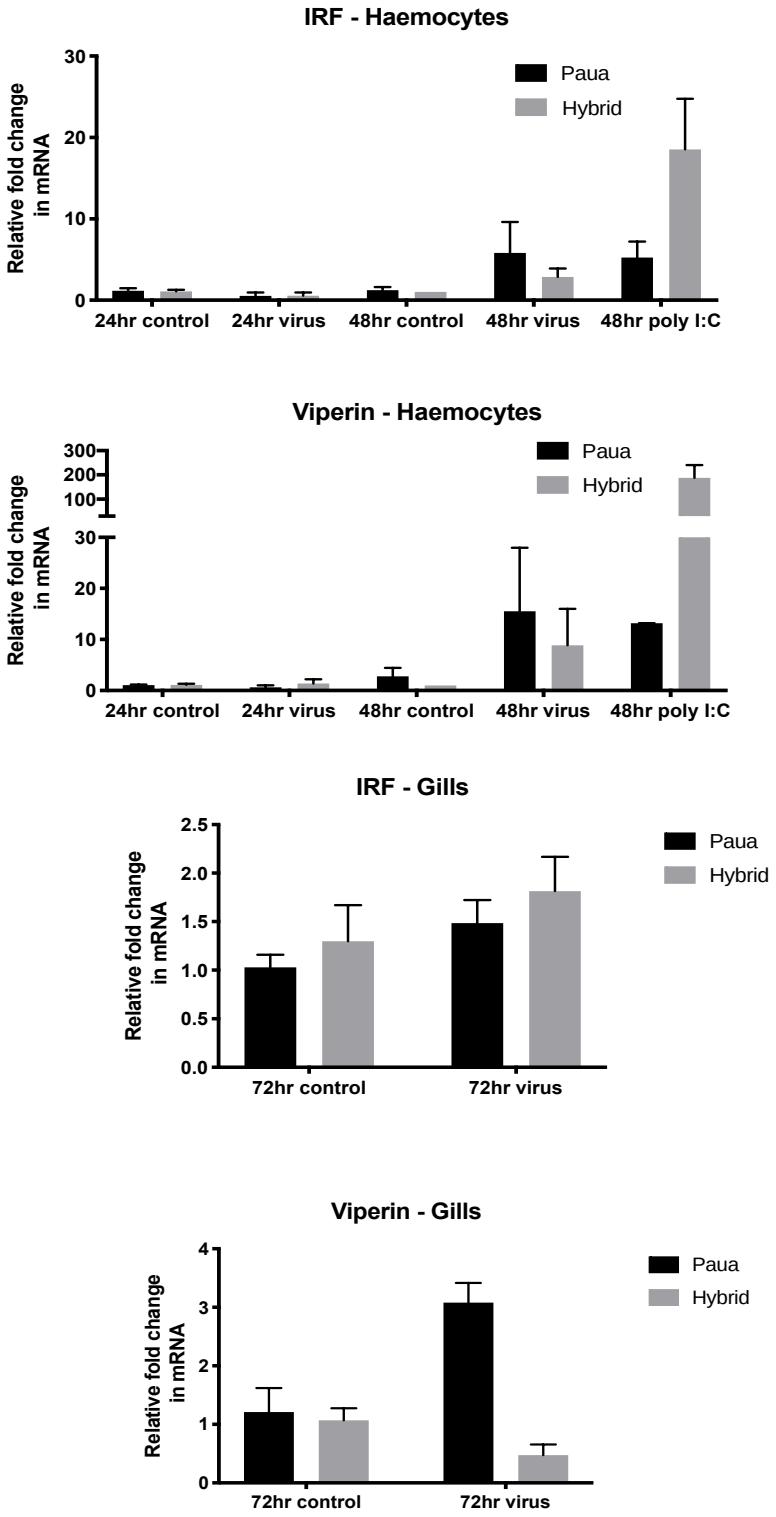



**Figure 1: Real-time PCR analysis of IRF-1/viperin across abalone species, tissue type and time point.**

**OBJECTIVES 2 and 3**

Through mRNA sequencing and genomic analysis, identify early genes expressed in päua and Australian hybrid abalone upon HaHV-1 immersion challenge and establish an immune signature in the early response of the host to the virus that differs between the päua and Australian hybrid abalone, to determine key immune players in HaHV-1 resistance.

Both objectives two and three were assessed utilising whole genome transcriptome analysis of päua and hybrid abalone species either treated with poly I:C (RNA viral mimic) or challenged with HaHV-1. Treatment of both abalone species with poly I:C was performed to initially assess the transcriptome response of abalone haemocytes to a viral mimic, without the opportunity for immune system interference by viral antagonists. The second set of transcriptional profiling assessed the immune response of both abalone species to HaHV-1 in two tissue type; haemocytes at 48 hours and gills at 72 hours post-challenge.

**Mapping Statistics**

The depth of sequencing (number of reads per sample) and mapping is presented in Appendix 1. The mapping performance was greater than 84% in all samples and demonstrated high mapping performance against the employed assembled genome for Red Abalone (*Haliotis rufescens*). This assembled abalone genome is the most recent, and following assessment of all available abalone genomes, was also the most completed. Additionally, all samples were shown to cluster according to tissue type and abalone species, as can be seen in the principal component analysis plot below (Figure 2). Given the extreme distance separation between the two abalone species during mock treatment from both tissue types, it was assumed that their basal transcriptome differed significantly.

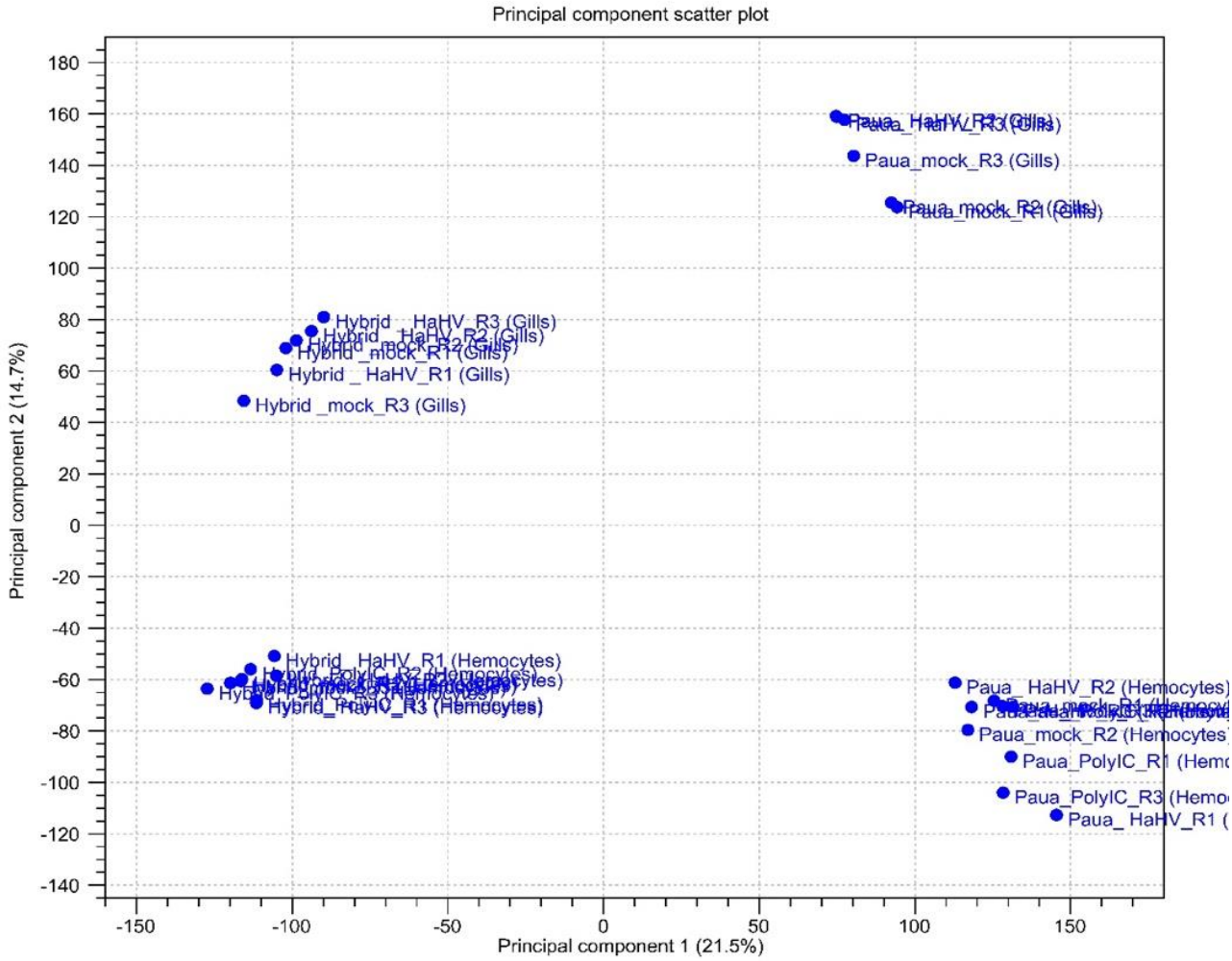


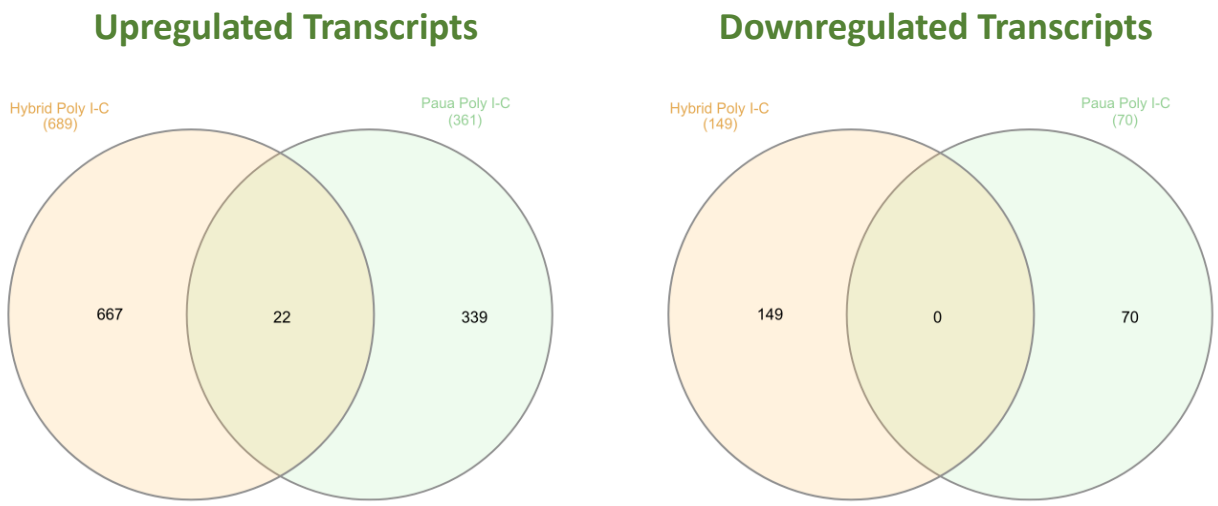

**Figure 2: Principal component scatter plot**

**Transcriptional profile of päua versus hybrid abalone to poly I:C**

When a host cell is infected with a virus, a number of internal sentinel receptors can detect the foreign nucleic acid, and this detection initiates an anti-viral response pathway, which ultimately upregulates a suite of anti-viral genes, some of which may code for secreted proteins. This overall response aims to protect the infected host cell, as well as neighbouring uninfected bystander cells. In order to examine this response pathway in the abalone for the first time and determine if it is similar between two abalone species, we intramuscularly injected a synthetic viral mimic (Poly I:C), which would initiate an anti-viral response from the animal tissues.

Following viral mimic stimulation of the two abalone species, a total of 689 and 361 transcripts were found to be upregulated in the haemocytes of the hybrid and the päua abalone species respectively, with 149 and 70 transcripts downregulated respectively (< or > 2.0 fold, FDR $p < 0.05$). Interestingly, there was very little overlap between the gene sets regulated between these two species following viral mimic stimulation, indicating that their initial response to a virus is likely to be significantly different (Figure 3).

**Figure 3: Differentially regulated transcripts following poly I:C stimulation**




The top 25 upregulated genes can be seen in Table 3.1 and Table 3.2. Many of the upregulated genes within the hybrid abalone are currently uncharacterised, and as such it is difficult to describe a trend in gene signature. However, in respect to the päua abalone, many of the early upregulated genes belong to extracellular matrix protein families, including multiple sub-species of collagen and matrillin (Table 3.2). It has been well documented in mammals that there is an intricate connection between a successful innate response to pathogens and extracellular matrix (Tomlin and Piccinini, 2018), and it is known that there is a transcriptional upregulation of extracellular matrix remodelling proteins during successful responses to pathogenic organisms in the oyster (McDowell et al., 2014). This extracellular matrix response was completely absent in the hybrid abalone at this time point, and in this tissue.

**Table 3.1 Top 25 upregulated genes in the hybrid abalone following poly I:C stimulation**

| Reference Gene ID | Fold change | Gene Name |
|---|---|---|
| Halref02648 | 1974 | uncharacterised protein |
| Halref39013 | 1376 | uncharacterised protein |
| Halref55116 | 1375 | TBC1 domain family member 5-like isoform |
| Halref17265 | 741 | uncharacterised protein |
| Halref46715 | 474 | uncharacterised protein |
| Halref16764 | 358 | cytochrome P450 3A2 |
| Halref31254 | 311 | uncharacterised protein |
| Halref15035 | 274 | extensin-1-like |
| Halref37879 | 159 | PREDICTED: sodium- and chloride-dependent GABA transporter 2-like |
| Halref08021 | 137 | PREDICTED: ras-like protein family member 12 |
| Halref06826 | 127 | uncharacterised protein |
| Halref42064 | 117 | uncharacterised protein |
| Halref39303 | 116 | uncharacterised protein |
| Halref45527 | 115 | sodium-coupled monocarboxylate transporter 1-like |
| Halref09174 | 115 | uncharacterised protein |
| Halref57523 | 112 | uncharacterised protein |
| Halref24459 | 110 | uncharacterised protein |
| Halref18757 | 107 | uncharacterised protein |
| Halref33338 | 100 | Protein of unknown function |
| Halref04919 | 97 | Protein of unknown function |
| Halref03892 | 97 | PREDICTED: fibropellin-1-like isoform X1 |
| Halref32789 | 93 | uncharacterised protein |
| Halref08264 | 93 | uncharacterised protein |
| Halref53481 | 89 | Ice nucleation protein InaA-like |
| Halref29144 | 84 | uncharacterised protein |

**Table 3.2 Top 25 upregulated genes in the päua following poly I:C stimulation**

| Reference Gene ID | Fold change | Gene Name |
|---|---|---|
| Halref30138 | 2148 | uncharacterised protein |
| Halref23359 | 1909 | uncharacterised protein |
| Halref23362 | 991 | zinc finger HIT domain-containing protein 3 isoform 3 |
| Halref30140 | 686 | collagen alpha-1(XXII) chain isoform X8 |
| Halref23361 | 444 | coiled-coil domain-containing protein 151 isoform 1 |
| Halref29612 | 442 | collagen alpha-1(XII) chain isoform X3 |
| Halref30136 | 190 | collagen alpha-1(XIV) chain isoform X2 |
| Halref23355 | 140 | serine protease 56 isoform 2 precursor |
| Halref53373 | 102 | collagen, type XXI, alpha 1 |
| Halref23354 | 97 | uncharacterised protein |
| Halref23353 | 88 | collagen alpha-1(XXII) chain isoform X8 |
| Halref30958 | 86 | uncharacterised protein |
| Halref38774 | 85 | matrilin-3 alternative transcript |
| Halref35468 | 84 | charged multivesicular body protein 1a isoform X1 |
| Halref02468 | 80 | uncharacterised protein |
| Halref02563 | 79 | collagen alpha-1(XXII) chain isoform X8 |
| Halref27759 | 78 | Chain A, SIGNAL-REGULATORY PROTEIN BETA 1 |
| Halref13300 | 77 | uncharacterised protein |
| Halref00342 | 65 | chorionic somatomammotropin |
| Halref13637 | 60 | stereocilin precursor |
| Halref45029 | 60 | heat shock cognate 71 kDa protein |
| Halref55316 | 59 | Chain A, Human Orexin receptor type 2 |
| Halref23365 | 58 | uncharacterised protein |
| Halref34873 | 53 | collagen, type XXI, alpha 1 |
| Halref13169 | 51 | inhibitor of apoptosis protein-1 |

**Transcriptional profile of päua versus hybrid abalone to HaHV-1**

Following HaHV-1 challenge of the abalone species, both the haemocytes (48hrs post-challenge) and gills (72 hours post-challenge) were analysed for their transcriptional profiles.

Analysis of the haemocytes revealed that a total of 299 and 194 transcripts were found to be upregulated in hybrid and the päua species respectively, with only 6 transcripts being common. A total of 104 and 81 transcripts were found to be downregulated in the hybrid and päua respectively following viral challenge, with only 1 common transcript (< or > 2.0 fold, FDR $p < 0.05$; Figure 4).

Analysis of the gills at 72 hours post-challenge of HaHV-1 in both abalone species displayed a similar trend as the haemocytes, with minimal overlap of either the upregulated or downregulated transcripts. There was a much greater transcriptional response in the gills compared to the haemocytes, which most likely represents both the diversity of cell types present in this tissue, as well as the later time point of RNA extraction. However, in contrast to the haemocytes, the päua was seen to upregulate and downregulate a substantially greater number of genes (2610 and 2463 transcripts respectively; < or > 2.0 fold, FDR $p < 0.05$; Figure 5), in comparison to the hybrid abalone gills samples which displayed 432 upregulated and 619 downregulated transcripts respectively (Figure 5). This significant difference in transcriptional response, may however be due to the tissue viral burden at this time point, with the hybrid abalone potentially beginning to shut down (hybrid abalone began to show significant signs of disease at 72 hours post-challenge).

**Figure 4: Differentially regulated transcripts in haemocytes 72h following HaHV-1 challenge**

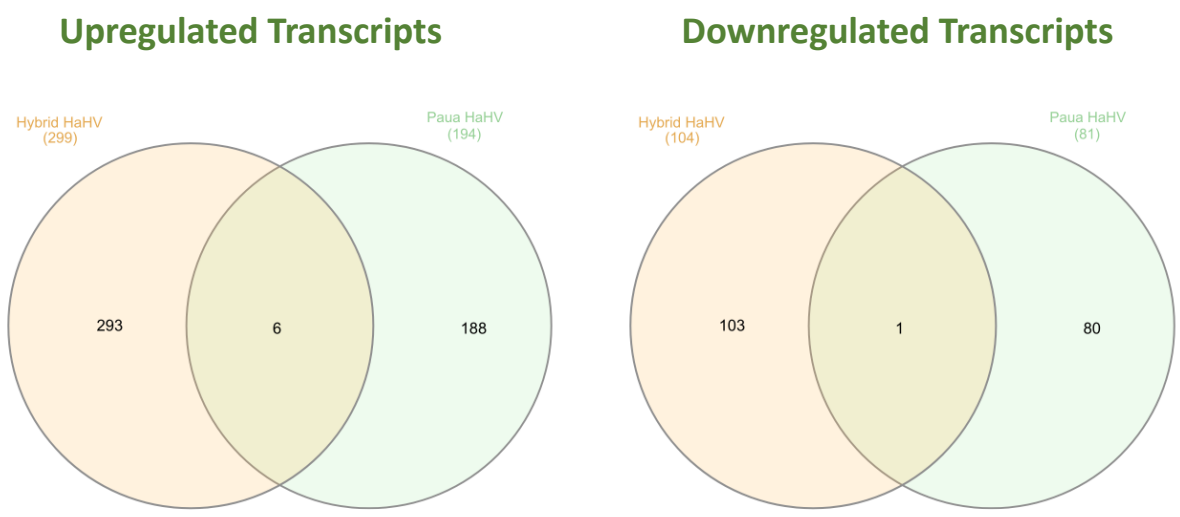
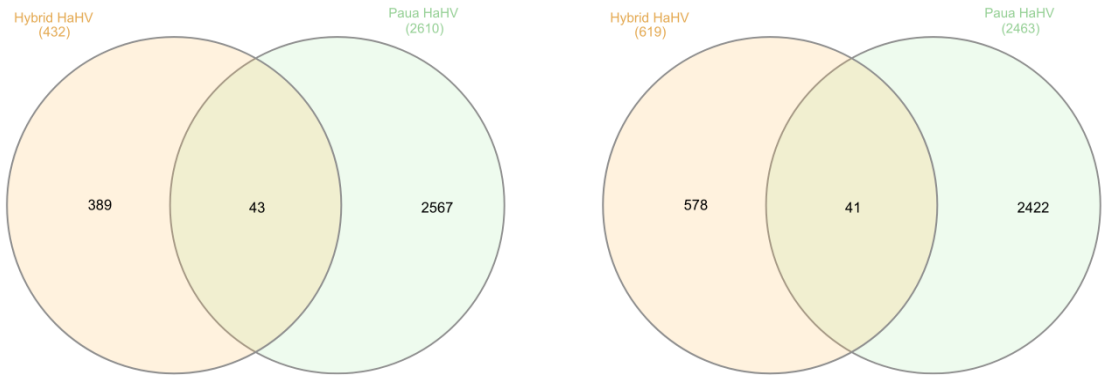



**Figure 5: Differentially regulated transcripts in the gills 72h following HaHV-1 challenge**




The top differentially upregulated genes can be seen in the below tables for both the hybrid and päua abalone, in both the haemocytes at 48 hours, and the gills at 72 hours post viral challenge. Once again, there was a dominant upregulation of transcripts known to be involved in remodelling the extracellular matrix in the päua abalone (Haemocytes, 48 hrs post HaHV-1 challenge, Table 4.2); including collagen, zonadhesin, adhesive plaque matrix protein-like, CUL3, and Plasminogen. Additionally, there were also numerous incidences of the upregulation of mariner transposases in the upregulated transcripts, which are functional enzymes involved in the ability of genetic material to reposition itself. These features were absent in the hybrid abalone transcriptional response to HaHV-1. Once again, the majority of the upregulated hybrid abalone transcripts were for uncharacterised protein products (Table 4.1).

The transcriptional response to HaHV-1 in the gills at 72 hours post-challenge (Table 4.3 and Table 4.4), was markedly different between the two species once again, as well as significantly different from the haemocytes within each abalone species. The hybrid abalone highest upregulated transcripts in response to HaHV-1 were related to matrix remodelling and were not upregulated at all in the päua gills at this time point. There were no other obvious gene transcript patterns present in the highest upregulated transcripts from these tissues.

**Table 4.1 Top 25 upregulated genes in the hybrid abalone following HaHV-1 challenge (Haemocytes)**

| Reference Gene ID | Fold change | Gene Name |
|---|---|---|
| Halref16719 | 1480 | D-arabinono-1,4-lactone oxidase-like |
| Halref18098 | 1464 | uncharacterised protein |
| Halref49370 | 844 | eukaryotic translation initiation factor 3 subunit C-like |
| Halref22185 | 640 | major vault protein-like |
| Halref06516 | 565 | P2X purinoceptor 7-like |
| Halref12150 | 553 | uncharacterised protein |
| Halref02027 | 538 | transmembrane protein 62-like |
| Halref03129 | 484 | uncharacterised protein |
| Halref09589 | 479 | calcium-dependent protein kinase 31-like |
| Halref48739 | 449 | diacylglycerol kinase delta-like isoform X3 |
| Halref26225 | 429 | Oxysterol-binding protein-related protein 9 |
| Halref37059 | 400 | calmodulin-like protein 5 |
| Halref36334 | 376 | Toll-like receptor f |
| Halref32437 | 310 | uncharacterised protein |
| Halref34297 | 257 | uncharacterised protein |
| Halref34645 | 176 | uncharacterised protein |
| Halref42933 | 152 | uncharacterised protein |
| Halref43588 | 136 | uncharacterised protein |
| Halref15090 | 135 | uncharacterised protein |
| Halref54101 | 134 | uncharacterised protein |
| Halref37153 | 112 | glycosyltransferase |
| Halref37160 | 106 | probable beta-D-xylosidase 7 |
| Halref40535 | 92 | uncharacterised protein |
| Halref26575 | 90 | plasminogen-like |
| Halref49973 | 87 | uncharacterised protein |

**Table 4.2 Top 25 upregulated genes in the päua abalone following HaHV-1 infection (Haemocytes)**

| Reference Gene ID | Fold change | Gene Name |
|---|---|---|
| Halref06172 | 828 | Coagulation factor V |
| Halref39746 | 685 | zonadhesin |
| Halref50451 | 182 | ADP-ribosylation factor 3 isoform X2 |
| Halref39902 | 144 | TatD DNase domain containing 2 |
| Halref44337 | 110 | cation-dependent mannose-6-phosphate receptor-like |
| Halref27999 | 73 | FAT tumor suppressor homolog 4 |
| Halref04517 | 38 | Similar to sorting nexin 13 |
| Halref37356 | 30 | adhesive plaque matrix protein-like |
| Halref40201 | 28 | transcription intermediary factor 1 |
| Halref09084 | 27 | collagen alpha-2(IV) chain-like |
| Halref04569 | 27 | glucuronosyltransferase I |
| Halref29326 | 23 | P-selectin glycoprotein ligand 1-like isoform X2 |
| Halref01981 | 22 | neuropeptide Y receptor type 2 |
| Halref53931 | 18 | BTB/POZ domain-containing protein KCTD7 isoform 2 |
| Halref23971 | 18 | maltase-glucoamylase, intestinal-like |
| Halref43045 | 16 | putative golgin subfamily A member 6-like protein 19 |
| Halref14358 | 16 | CUL3 protein |
| Halref23969 | 15 | mariner transposase |
| Halref00342 | 14 | core-binding factor, beta subunit |
| Halref55509 | 14 | Chain A, Plasminogen |
| Halref29453 | 13 | UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransfera |
| Halref47122 | 13 | mariner transposase |
| Halref44984 | 12 | calcium-independent phospholipase A2-gamma-like |
| Halref24923 | 12 | CD40 antigen (TNF receptor superfamily member 5) |
| Halref20691 | 12 | EGF-like-domain, multiple 4 |
| Halref29774 | 12 | ribonuclease H1 isoform 2 |
| Halref03350 | 11 | EGR1 |

**Table 4.3 Top 25 upregulated genes in the hybrid abalone following HaHV-1 challenge (Gills)**

| Reference Gene ID | Fold change | Gene name |
|---|---|---|
| Halref41591 | 8042 | adhesive plaque matrix protein-like |
| Halref41590 | 3231 | adhesive plaque matrix protein-like |
| Halref13851 | 2386 | high affinity cationic amino acid transporter 1-like |
| Halref05976 | 2235 | protein CNPPD1 |
| Halref18098 | 2143 | uncharacterised protein |
| Halref03404 | 2033 | uncharacterised protein |
| Halref51737 | 1818 | uncharacterised protein |
| Halref18099 | 1450 | uncharacterised protein |
| Halref24942 | 1144 | cilia- and flagella-associated protein 44-like |
| Halref00160 | 969 | PREDICTED: DNA-directed RNA polymerase II subunit RPB3-like |
| Halref24071 | 957 | cytochrome P450 27C1-like isoform X2 |
| Halref44381 | 952 | uncharacterised protein |
| Halref30152 | 751 | uncharacterised protein |
| Halref14342 | 708 | cilia- and flagella-associated protein 44-like isoform X1 |
| Halref40078 | 651 | Paraneoplastic antigen Ma2-like |
| Halref10861 | 580 | uncharacterised protein |
| Halref18100 | 569 | uncharacterised protein |
| Halref24490 | 522 | DNA-directed RNA polymerase II subunit RPB3-like |
| Halref03738 | 516 | PREDICTED: periaxin-like |
| Halref45650 | 515 | dishevelled |
| Halref46391 | 492 | Glycine, alanine and asparagine-rich protein; Flags: Precursor |
| Halref11836 | 460 | Perlwapin |
| Halref00600 | 440 | sodium-coupled monocarboxylate transporter 1-like |
| Halref37927 | 434 | uncharacterised protein |
| Halref16651 | 422 | Paraneoplastic antigen-like protein 5 |

**Table 4.4 Top 25 upregulated genes in the päua abalone following HaHV-1 challenge (Gills)**

| Reference Gene ID | Fold change | Gene name |
|---|---|---|
| Halref41372 | 1725 | uncharacterised protein |
| Halref32094 | 946 | uncharacterised protein |
| Halref16908 | 829 | uncharacterised protein |
| Halref28983 | 803 | uncharacterised protein |
| Halref17210 | 643 | putative aminopeptidase W07G4.4 isoform X1 |
| Halref07654 | 613 | histone deacetylase complex subunit SAP30L-like |
| Halref03282 | 593 | histone deacetylase complex subunit SAP30L-like |
| Halref41375 | 510 | uncharacterised protein |
| Halref56392 | 434 | fibrillin-1-like isoform X1 |
| Halref23030 | 395 | deoxyribonuclease-1 |
| Halref04504 | 356 | uncharacterised protein |
| Halref35570 | 349 | multiple epidermal growth factor-like domains protein 10 |
| Halref45952 | 344 | uncharacterised protein |
| Halref43339 | 339 | receptor-type tyrosine-protein phosphatase kappa-like |
| Halref28462 | 297 | arylsulfatase B-like |
| Halref46733 | 297 | arylsulfatase B-like |
| Halref16240 | 285 | uncharacterised protein |
| Halref35141 | 280 | uncharacterised protein |
| Halref45455 | 279 | ras-related and estrogen-regulated growth inhibitor-like |
| Halref00758 | 261 | TATA element modulatory factor-like isoform X2 |
| Halref09818 | 252 | tRNA (guanine-N(7)-)-methyltransferase non-catalytic subunit |
| Halref56215 | 243 | PREDICTED: poly [ADP-ribose] polymerase 14-like |
| Halref36816 | 221 | uncharacterised protein |
| Halref47417 | 218 | mucin-5AC-like |
| Halref28554 | 218 | PREDICTED: fibroblast growth factor receptor 2-like |

# Conclusion

The principal aim of this project was to identify differential gene expression in AVG resistant päua (*H.iris*) and susceptible hybrid abalone upon HaHV-1 challenge using RNA sequencing and gene analysis. Preliminary testing using the immune genes viperin and IRF-1 as targets allowed us to identify favourable tissues and time points after challenge to perform RNA sequencing on genes expressed in both species.

The transcriptome profiling of the two abalone species was performed against both a dsRNA viral mimic to assess what a normal response to a viral infection would look like in the absence of viral antagonism in each abalone species, as well as following HaHV-1 viral challenge. The overarching result of the RNA sequencing was that the early anti-viral response of the two abalone species is very different to both a synthetic viral mimic as well as HaHV-1. There was less than 5% transcript overlap in most cases, indicating a very different anti-viral immune response pathway in each animal. However, it cannot be excluded that the hybrid abalone may have a more delayed immune response time, and that this may account for some of the transcriptional response difference between species; however, this would be unlikely to completely account for the noticeable differences observed. Additionally, it should also be noted that molecular phylogenetic studies demonstrate that the Australian abalone species fall within an alternate clade to *H.iris*, and that this may also account for some of the transcript profile differences observed. However, the early host response to viral infection in multiple different animals is known to contain core sets of anti-viral genes, and it remains to be determined if this would be the case in shellfish.

The päua abalone appears to have a strong transcriptional response linked to matrix remodelling in the haemocytes following both stimulation with a viral mimic, as well as following challenge with HaHV-1 which is in line with results from a recent study published by Neave et al. (2019). This response appears to be absent in the haemocytes of the hybrid abalone, however there was some indication that a matrix-remodelling response may be occurring in the gill transcriptome at a later time point, potentially adding weight to the hypothesis that the hybrid abalone may have a more delayed anti-viral response. It should however also be noted that many differentially regulated transcripts could not be identified in both species of abalone, and this is most likely due to the novel nature of the abalone genomes, and the lack of information and research surrounding analysis of gene function in most marine organisms.

Collectively the work from this project has optimised real-time PCR in an abalone species, and across abalone species for the first time. It has demonstrated that two abalone species can have a very divergent transcriptional response to a viral pathogen; and that the HaHV-1 resistant päua abalone has a heightened transcriptional response that demonstrates an upregulation of genes involved in remodelling of matrix, which appears to be absent in the hybrid abalone at similar time points.

# Implications and further development

AVG remains a serious concern to all State jurisdictions with significant commercial investments in abalone fisheries and aquaculture. Data obtained from this project will be submitted to peer-reviewed journals for publication and will provide the basic knowledge on the mechanisms of resistance to AVG.

The knowledge gained from this study gives us insight for the first time into the anti-viral transcriptome response of a HaHV-1 resistant abalone species, and allows us to begin to determine which pathways and/or genes may be involved in a successful host abalone response to viral infection.

Future work could focus on determining the genes responsible for specific protection against AVG. In order to demonstrate which genes have a major functionality in the protective mechanisms, the silencing (or knock down of expression) of each one individually, and/or many in combination, would confirm the presumptive role of these genes highlighted in this current genomic study. The RNAi technology, widely used in various species including abalone, has proven effective in silencing/knocking down gene expression and demonstrating their function in animal and plant species. The application of such technology could provide the information on selected genes in the päua with regards to AVG resistance. Multiple avenues could then

be taken to attempt to facilitate a similar response in the Australian hybrid abalone, including (1) determining the ability of Australian hybrid abalone to upregulate these genes in response to certain stimuli to develop a 'vaccine like' strategy; (2) developing a high through-put screening system to screen Australian hybrid abalone for a 'HaHV-1 resistant-like profile, and initiate breeding programs based on this knowledge or (3) exploring gene knock-in using CRISPR/cas technology; which has been performed successfully in fish, but is a long-term prospect.

# Recommendations

Numerous further studies could be implemented from the data presented within this report including:

1. The early up-regulation of the matrix remodelling gene and collagen genes in the päua upon poly-I:C injection as well as viral challenge and the absence or delayed up-regulation in the hybrid abalone is suggestive of their involvement in the protection against AVG. In order to confirm this hypothesis it would be beneficial to perform further studies using cutting edge technologies such as gene editing or RNA interference to eliminate or reduce the function of these genes in vivo and proceed to a live challenge of päua 'deprived' of these genes. If the importance of the matrix remodelling and collagen genes was confirmed, such families of genes could be targeted in a breeding program for AVG resistance.

2. There is now published genomes for both the hybrid and päua abalone; and this project has generated de novo transcriptomes for both genomes for the first time. Both genomes should be screened for the presence of presumed essential and known viral pattern recognition receptors, and potential known signalling adaptor proteins, to ensure that the hybrid and päua abalone species have a similar complement of known anti-viral signalling molecules. Major differences may point to potential targets for breeding programs, as well genetic engineering opportunities of the hybrid abalone.

3. The päua abalone response to a synthetic viral mimic is now known, and a similar response in the hybrid abalone could now be therapeutically sought as a priming protective strategy to protect Australian abalone against HaHV-1; as has been employed in the oyster against OsHV-1 successfully.

# Further development

Future work currently underway will also investigate the main biological pathways upregulated in both abalone species in response to viral mimics as well as challenge with HaHV-1; this may shed further information on the essential pathways required for a successful abalone response to immune challenge.

# Extension and Adoption

This project has generated de novo transcriptomes for the greenlip x blacklip abalone and päua genomes for the first time and this data will be made available to the scientific community through deposition of the sequences at a relevant genome databank.

**Presentation of results at conferences;**

Sarker S, Ebrahimie E, Corbeil S,Helbig K. The abalone viral ganglioneuritis resistant päua (*Haliotis iris*) displays a highly divergent tissue transcriptome profile in respect to the susceptible Australian *Haliotis rubra* x *H. laevigata* hybrid abalone. Australasian Virology Society Meeting (accepted paper) Queenstown, NZ 2-5 Dec 2019.

Serge Corbeil, Subir Sarker, Karla Helbig. Evaluate and compare the gene expression in AVG resistant paua and AVG susceptible hybrid abalone (greenlip x blacklip) upon immersion challenge with HaHV-1. AAGA workshop in Hobart 01 August 2019

Serge Corbeil, Subir Sarker, Karla Helbig. Evaluate and compare the gene expression in AVG resistant paua and AVG susceptible hybrid abalone (greenlip x blacklip) upon immersion challenge with HaHV-1. 5th FRDC Australasian Conference on Aquatic Animal Health & Biosecurity. Cairns, Qld Australia 8-12 July 2019.

Corbeil S. Identification of differentially expressed innate immune genes in the NZ paua (*Haliotis iris*) and the Australian hybrid abalone (*H. laevigata* X *H. rubra*) upon immersion challenge with Haliotid herpesvirus-1 (HaHV-1). AAGA workshop, Queenscliff, Vic 09-08-2018.

# Project coverage

See above

# Project materials developed

Scientific manuscripts will be submitted to international peer-reviewed journals for publication.

# Appendices

### Appendix 1: Mapping statistics

Summary of mapping statistics of samples against *Haliotis rufescens* (Red Abalone) assembled genome

| Sample name | Read count | Mapped % |
|---|---|---|
| 3_115_CDNJBANXX_ACAGTGA | 37099450 | 94.08 |
| 9_133_CDNJBANXX_ATGTCAG | 37567944 | 88.86 |
| 1_111_CDNJBANXX_CGATGTA | 44735402 | 94.37 |
| 11_121_CDNJBANXX_GTCCGCA | 30459562 | 87.99 |
| 10_119_CDNJBANXX_CCGTCCC | 37337018 | 88.62 |
| 16_65_CDNJBANXX_GATCAGA | 34722436 | 89.88 |
| 25_81_CDNJBANXX_AGTCAAC | 36842424 | 84.03 |
| 5_107_4_103_CDNJBANXX_CAGATCA | 73731820 | 94.72 |
| 8_129_CDNJBANXX_AGTTCCG | 34487692 | 88.36 |
| 21_89_CDNJBANXX_ACAGTGA | 38731788 | 90.86 |
| 26_82_CDNJBANXX_AGTTCCG | 32270370 | 86.96 |
| 15_94_CDNJBANXX_ACTTGAA | 32685336 | 87.67 |
| 20_88_CDNJBANXX_TGACCAA | 33608030 | 93.02 |
| 27_83_CDNJBANXX_ATGTCAG | 32377538 | 86.26 |
| 7_127_CDNJBANXX_AGTCAAC | 40568432 | 89.4 |
| 14_92_CDNJBANXX_TTAGGCA | 31964720 | 85.92 |
| 13_91_CDNJBANXX_ATCACGA | 32921898 | 88.53 |
| 22_70_CDNJBANXX_GCCAATA | 31074858 | 91.74 |
| 28_84_CDNJBANXX_CCGTCCC | 31327706 | 84.64 |
| 19_87_CDNJBANXX_CGATGTA | 35039654 | 86.13 |
| 6_109_CDNJBANXX_CTTGTAA | 39296678 | 94.08 |
| 12_123_CDNJBANXX_GTGAAAC | 31726652 | 87.23 |
| 2_113_CDNJBANXX_TGACCAA | 37476378 | 93.71 |
| 17_67_CDNJBANXX_TAGCTTA | 38212292 | 90.4 |
| 24_72_CDNJBANXX_CTTGTAA | 34334588 | 91.42 |
| 5_107_4_103_CDNJBANXX_CAGATCA | 35000000 | 94.71 |
| 18_68_CDNJBANXX_GGCTACA | 39081516 | 87.87 |
| 29_85_merged | 29723100 | 89.57 |
| 30_86_CDNJBANXX_GTGAAAC | 31311324 | 87.01 |
| 23_71_CDNJBANXX_CAGATCA | 35892576 | 89.72 |
| 4_103_CDPFRANXX_CAGATCA | 36960314 | 94.66 |

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## Intellectual Property

No intellectual property has been identified.

# References


Bai CM, 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 Invert Pathol 160: 26-32.

Chang PH, Kuo ST, Lai SH, Yang HS, Hsu CL, Chen HC (2005) Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertaxa* in Taiwan. Dis Aquat Org 65: 23-27.

Corbeil S, McColl KA, Williams LM, Mohammad I, Hyatt AD, Crameri SG, Fegan M, Crane MSt-J (2012a) Abalone viral ganglioneuritis: Establishment and use of an experimental immersion challenge system for the study of abalone herpes virus infections in Australian abalone. Virus Res 165: 207-213.

Corbeil S, Williams LM, Bergfeld J, Crane MSt-J (2012b) Abalone herpes virus stability in sea water and susceptibility to chemical disinfectants. Aquaculture 326-329: 20-26.

Corbeil S, Williams LM, McColl KA, Crane MSt-J (2016) Australian abalone (*Haliotis laevigata*, *H. rubra* and *H. conicorpora*) are susceptible to infection by multiple abalone herpesvirus genotypes. Dis Aquat Org 119:101-106. doi: 10.3354/dao02989

Corbeil S, McColl KA, Williams LM, Slater J, Crane M StJ (2017) Innate resistance of New Zealand päua to abalone viral ganglioneuritis. J Invert Pathol 146:31-35. doi.org/10.1016/j.jip.2017.04.005

Crane MSt-J, Corbeil S, Williams LM, McColl KA, Gannon V, (2013) Evaluation of the abalone viral ganglioneuritis resistance among wild abalone populations along the Victorian coast of Australia. J Shell Res 32(1): 67-72.

Green TJ, Speck P, Geng L, Raftos D, Beard MR, Helbig KJ (2015a) Oyster viperin retains direct antiviral activity and its transcription occurs via a signalling pathway involving a heat-stable haemolymph protein. J Gen Virol 96:3587-3597. DOI 10.1099/jgv.0.000300

Helbig KJ, Beard MR (2014) The role of viperin in the innate antiviral response. J Mol Biol 426:1210-1219.

Masonbrink RE, Purcell CM, Boles SE, Whitehead A, Hyde JR, Seetharam A, Severin AJ (2019) An Annotated Genome for Haliotis rufescens (Red Abalone) and Resequenced Green, Pink, Pinto, Black, and White Abalone Species. Genome biology and evolution 11, 431-438.

McDowell IC, Nikapitiya C, Aguiar D, Lane CE, Istrail S, Gomez-Chiarri M (2014) Transcriptome of American Oysters, Crassostera virginica, in response to bacterial challenge: Insights into potential mechanisms of disease resistance. Plos one 9: e105097.

Neave M, Corbeil S, McColl KA, Crane MSt-J (2019) Investigating the natural resistance of blackfoot päua *Haliotis iris* to abalone viral ganglioneuritis using whole transcriptome analysis. Dis Aquat Org doi: 10.3354/dao03390

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139-140.

Tomlin H, Piccinini AM (2018) A complex interplay between the extracellular matrix and the innate immune response to microbial pathogens. Immunology 155: 186-201.

Wang JY, Guo ZX, Feng J, Liu GF, Xu LW, Chen BS, Pan JP (2004) Virus infection in culture abalone, *Haliotis diversicolor* Reeve in Guandong province China. J Shellfish Res 23(4): 1163-1168.