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# Population genomic assessment of Australian Blacklip Abalone for Abalone viral ganglioneuritis (AVG) resistance

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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

AVG – Abalone viral ganglioneuritis

ACDP- Australian Centre for Disease Preparedness

BF – Bayes factor

BP- base pair

CRISPR - Clustered regularly interspaced short palindromic repeats

dB - Deciban units

DEDJTR - Department of Economic Development, Jobs, Transport and Resources

DNA - Deoxyribonucleic acid

$F_{ST}$ - Fixation index

Gb - Giga base

GWAS - Genome-wide association study

HaHV-1 - Haliotid herpesvirus-1

LD - Linkage disequilibrium

SNP – Single nucleotide polymorphism

# Executive Summary

Abalone viral ganglioneuritis (AVG) remains a significant threat to the economic viability and stability of the Abalone industry in south-eastern Australia. Consequently, there is an urgent need for strategic research aimed at determining the likely vulnerability of fisheries to future AVG outbreaks and providing managers with the necessary tools for biosecuring wild and farmed stocks at regional, state and national scales. The fact that some animals from AVG affected wild stocks survived the disease outbreak in the early 2000s suggests they were either fortunate enough to have avoided coming into contact with the virus or are genetically resistant to the disease. A research program aimed at characterising AVG resistance in Australian wild Abalone fisheries is expected to provide benefits to wild and farm fisheries at a national scale. If AVG resistance is present in wild Abalone stocks, and its genetic basis can be characterised, there may be opportunities to:

1) Improve industry knowledge of the mechanisms for animal persistence in virus affected Abalone fisheries and on the resilience of Abalone fisheries to environmental change;
2) Screen wild fishing stocks across all wild fisheries to determine the spatial prevalence of resistant genotypes and to help identify stocks are likely to be resilient or vulnerable to AVG re-emergence;
3) Explore options for biosecuring wild stocks through the movement of AVG resistant genotypes to 'AVG vulnerable' stocks as part of future restocking and translocation activities; and
4) Establish AVG resistant breeding lines for biosecuring farm stocks across all states of Australia.

In this study we performed a genome wide association study on the Blacklip Abalone (*Haliotis rubra*) using pooled whole genome re-sequencing data from 343 *H. rubra* specimens representing 14 Victorian fishing stocks varying in historical AVG exposure. Analyses identified approximately 25,000 SNP loci associated with AVG exposure, many of which mapped to genes known to be involved in herpesvirus response pathways and general virus-host interactions in Haliotids and other animal systems. Most notably, candidate loci mapped to 24 genes known to be associated with Haliotid herpesvirus-1 (HaHV-1)

immunity in the New Zealand pāua (*H. iris*). Experimental trials are now needed to validate if, and how much, resistance is determined by the candidate genotypes identified in this study before the industry can harness them for management purposes with confidence. This study provides the first line of evidence of genetic changes and adaptive responses in AVG affected *H. rubra* fisheries. These findings have potential implications for future management which are discussed in detail but will require further investigation.

**Keywords**

Abalone viral ganglioneuritis (AGV), Haliotid herpesvirus-1 (HaHV-1), Blacklip Abalone, virus resistance, divergent adaptation, whole genome resequencing, genome wide association study.

# Introduction

In the Southern Ocean of Australia, the world's largest wild Abalone fisheries target Blacklip Abalone (*Haliotis rubra*) in five states extending from Western Australia to Tasmania and southern New South Wales, with a net value of US$79 million (Mundy et al. 2014). This is complemented by farmed Abalone produced by a rapidly expanding aquaculture industry which makes up approximately 10% of Australia's export market. Blacklip, Greenlip (*Haliotis laevigata*), and hybrid (*H. rubra* x *H. laevigata*) Abalone are commercially farmed, producing an estimated total volume of ~1,100 tonnes with a value of $37 million in 2016 (ABARES 2018). The most profitable wild and farm Abalone fisheries occur in south-eastern Australia but were heavily impacted by disease between 2006 and 2010. Specifically, Abalone viral ganglioneuritis (AVG) caused by the Haliotid herpesvirus-1 (HaHV-1) spread along the western coastline of Victoria, devastating wild and farmed Abalone stocks by causing high animal mortality (up to 90% in some areas) (Hooper et al. 2007; Mayfield et al. 2011; Figure 1). Wild and farm fisheries have taken many years to recover from these losses (Mayfield et al. 2011). Despite AVG not being recorded in Victoria waters since 2010, it still remains a significant threat to the economic viability of the industry in south-eastern Australia.

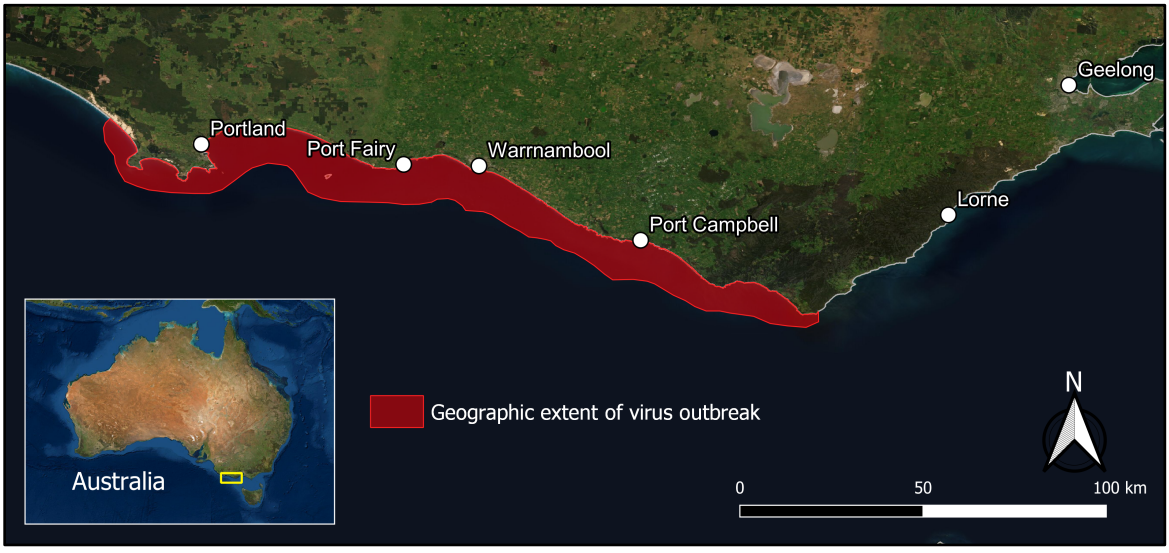


**Figure 1.** Map showing the known geographical extent of the Abalone viral ganglioneuritis outbreak along the Victorian coastline (indicated by red shading). Source: modified from Crane *et al.* 2013.

Some Abalone from affected stocks survived the disease outbreak but the mechanism(s) underpinning animal persistence remain largely uncertain. Evidence suggests that horizontal transmission (direct contact between infected and susceptible animals) is the primary mode of AVG infection, although virus transmission directly through the water column has also been experimentally demonstrated (Hardy-Smith 2006; Crane et al. 2013). It is likely that some animals avoided exposure to the virus, including solitary animals, isolated animal aggregations, and cryptic juvenile life stages. However, it is also likely that at least some surviving animals were genetically resistant to the disease, persisting after selection occurred. Environmental disturbances that have pronounced and rapid impacts on local population sizes, often impose significant selection pressure on local gene pools (Hoffmann & Sgro 2011). In such cases, animal persistence is often dictated by genotypes that provide a significant fitness advantage; the process of natural selection (Hedrick 2009). Consequently, the impact of AVG might have selected for virus resistant genotypes across affected Victorian fisheries.

Testing for AVG resistance in wild Abalone populations has been prioritised by industry as an important step towards improving the biosecurity of Australian wild and farm Abalone fisheries. If resistance is found in wild fisheries, managers could potentially characterise AVG vulnerable or resistant wild fishing stocks by mapping the spatial distribution and frequency of genotypes that control for resistance. Managers could also explore opportunities for enhancing base levels of resistance in vulnerable wild fisheries via the controlled introduction of AVG resistant genotypes through strategic restocking and translocation programs. The availability of AVG resistant animals could also greatly assist in biosecuring Australian farm stocks by providing opportunities for the establishment of AVG resistance breeding program(s). At present significant biosecurity investments are being made across Abalone farm fisheries to safeguard Abalone stocks from the re-emergence of AVG (Gavine et al. 2009; Department of Agriculture 2014). Australian Abalone farm fisheries have established breeding programs focussed on the selection of commercially important traits (primarily

relating to growth) and expanding these programs to accommodate AVG resistant breeding lines is recognised as a priority investment area for biosecuring farm stocks.

Understanding the mechanism for animal perseverance following the AVG outbreak (i.e. avoidance and/or genetic resistance) is also necessary for enhancing knowledge on *H. rubra*'s ability to respond and adapt to new environmental conditions. This is pertinent given the current and predicted impacts of climate change to the physical marine environment, ecosystem structure and function, and biotic interactions (Johnson et al. 2011; Pecl et al. 2014; Babcock et al. 2019). Previous population genomic work on *H. rubra* has indicated significant genotype associations with heterogeneous habitat features at local and regional spatial scales, suggesting adaptive genetic differentiation among Abalone stocks and the presence of standing genetic variation for adaptation to environmental changes (Miller et al. 2019). Evidence of evolutionary responses to AVG exposure will provide valuable new insights into the adaptability of *H. rubra* and the presence of genetic variation for countering environmental changes associated with disease.

At present, the occurrence of AVG resistance in wild populations of *H. rubra* remains uncertain. Challenge tests performed on New Zealand pāua (*Haliotis iris*) by the Australian Centre for Disease Preparedness (ACDP), involving controlled exposure to Haliotid herpesvirus-1 (HaHV-1), indicate complete resistance to AVG (Corbeil et al. 2017). Similar tests on *H. rubra* yielded no evidence of resistance to various AVG strains but were performed only on a small number of animals from a limited number of locations from AVG affected fisheries (Crane et al. 2012; Crane et al. 2013; Corbeil et al. 2016). Consequently, it remains unclear if AVG resistance persists in *H. rubra* and has spread through some natural populations following virus exposure.

In this study we build on previous research by testing for signatures of virus resistance in Victorian *H. rubra* fisheries using a population genomic approach. Specifically, we conducted a genome-wide association study using pooled whole genome re-sequencing data from 343 *H. rubra* specimens collected from 14 Victorian fishing stocks varying in historical AVG exposure. We directly

test for genotype associations with AVG exposure; indicators of divergent adaptation in AVG affected fishing stocks, and selection for genotypes that might contribute to virus resistance. This study provides the first insights into the evolutionary response of *H. rubra* to AVG exposure, the genetic complexity of AVG resistance, and the distribution of putatively resistant genotypes in AVG affected fisheries. Further quantitative experiments will be needed to validate the findings from this study; however, this is an important first step in identifying populations with potential AVG resistance in Victorian wild *H. rubra* fisheries.

# Objectives

The objective of this study is to:

1) Conduct a genome-wide association study on *H. rubra* to test for rapid evolutionary responses and genomic signatures of virus resistance in AVG affected Victorian fishing stocks. It is hoped that genomic variants associated with AVG exposure, potentially contributing to virus resistance, could act as the valuable biomarkers for surveying the resilience of wild fishing stocks to future AVG outbreaks, and biosecuring wild and farm stocks through strategic stocking and breeding programs.

# Methods

### *Sample collection and DNA sequencing*

Tissue biopsies were collected from a total of 343 individual *H. rubra* from 14 locations spanning the Victorian Western and Central Zone fisheries (Table 1, Figure 2). Locations were selected based on their known virus exposure history according to confidential records held by the Victorian wild fishing

sector and the Victorian Fisheries Authority (10 AVG affected and 4 AVG unaffected locations). Sampling for the majority of the locations within the Western Zone Fishery was coordinated in 2009 by the Department of Economic Development, Jobs, Transport and Resources (DEDJTR). It is expected that animals from these locations during this sampling period survived the virus event and provide a reliable snap shot of the post-virus allele frequency distributions in AVG affected and unaffected populations. To avoid the potential swamping effects of inter-generational gene flow since the virus event, sampling between 2015 and 2020 was biased towards fishing stocks expected to be largely self-recruiting based on available biophysical connectivity models (FRDC project *2015-025*: *Patterns of interaction between habitat and oceanographic variables affecting the connectivity and productivity of invertebrate fisheries*) and sampling was biased toward large adult animals (expected to be either direct survivors or first generation post-virus survivors). This sampling was performed by contract divers, commercial fisherman, and by our own research team. At each location, individual Abalone were collected within a 100 $m^2$ area, with tissue biopsies consisting of 20 mg of muscle tissue from the Abalone lip obtained using sterile dissection tools to avoid sample cross-contamination. Biopsied material was transferred to 2 ml microcentrifuge tubes containing 80-100% ethanol and stored at 4°C until required for genomic analysis.

**Table 1.** Site location details and corresponding codes for 14 collection locations of *Haliotis rubra* from Victorian Western and Central Zone fisheries. Sample sizes and AVG exposure history are also provided.

| Zone and Location | Code | Year Sampled | Sample Size | GPS Location | | AVG Status |
|---|---|---|---|---|---|---|
| | | | | Latitude | Longitude | |
| ***Western Zone fishery*** | | | | | | |
| Port Macdonell | **PMC** | 2020 | 25 | –38.054 | 140.881 | unaffected |
| Inside Murrels | **MUR** | 2009 | 25 | –38.407 | 141.524 | affected |
| Inside Nelson | **ISN** | 2009 | 25 | –38.409 | 141.558 | affected |
| The Craggs | **CRG** | 2009 | 25 | –38.390 | 142.135 | affected |
| Lady Julia Percy | **LJP** | 2009 | 23 | –38.422 | 141.993 | unaffected |
| Killarney | **KIL** | 2015 | 20 | –38.363 | 142.321 | affected |
| Levies | **LEV** | 2009 | 25 | –38.385 | 142.235 | affected |
| ***Central Zone fishery*** | | | | | | |
| Childers Cove | **CHC** | 2019 | 25 | –38.490 | 142.672 | affected |
| Bay of Islands | **BIP** | 2019 | 25 | –38.582 | 142.827 | affected |
| Cat Reef | **CAT** | 2015 | 25 | –38.741 | 143.188 | affected |
| White Cliffs | **WCF** | 2015 | 25 | –38.758 | 143.330 | affected |
| Castle Cove | **CCV** | 2020 | 25 | –38.783 | 143.422 | unaffected |
| Parker River | **PKR** | 2020 | 25 | –38.855 | 143.538 | affected |
| Blanket Bay | **BLK** | 2015 | 25 | –38.827 | 143.586 | unaffected |

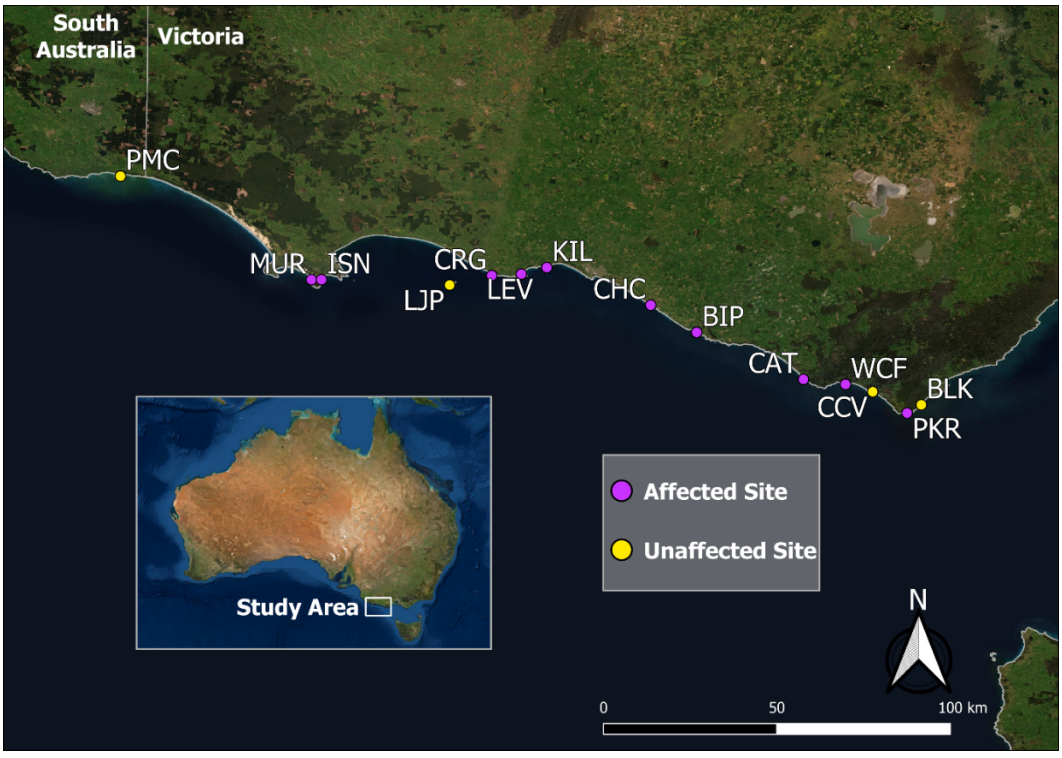


**Figure 2.** Sampling sites selected for population genomic analysis from the south-eastern Australia. Figure legend and colour coding of mapped sites indicate history of virus exposure. Refer to Table 1 for sample codes.

Total genomic DNA was extracted from 10 mg of tissue using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufactures instructions. Resulting DNA extracts were quantified using a Qubit version 2 fluorometer (Life Technologies, Carlsbad, CA, USA). To obtain population genomic data, we applied the Pool-Seq approach (Futschik & Schl€otterer 2010), which is based on pooling the DNA of a large number of individuals from the same population and then sequencing what can be paraphrased as a 'population variability genome'. This was achieved by pooling individual DNA extracts from each sample location equimolar, splitting the 25 individuals per location into 2 x pools per locations consisting of DNA from 12 and 13 individuals, respectively, to account for potential sequencing bias. The resulting 28 pooled libraries were prepared for sequencing using the NexteraTM DNA Sample Preparation kit (Nextera, USA), and sequenced using the Illumina NovaSeq platform (Illumina, San Diego, CA, USA), with the 150 base pair (bp) paired-end protocol. Sequencing was performed allowing for 3 x genome coverage (~ 1.3 giga base (Gb) genome; Gan *et al.* 2019) per individual per pool.

### *Data preparation*

The Illumina NovaSeq sequencing yielded approximately 25 x $10^9$ assigned 150 bp reads, and a total of 45 – 100 Gb of sequence data for each of the 28 pooled DNA libraries. Raw DNA sequence reads from the two separate pooled libraries per sample location were pooled for processing purposes. Raw sequences were processed using the Trimmomatic V0.36 program (Bolger et al. 2014) by removing Nextra adaptors and discarding all reads that had a Phred score below 20. All retained reads were subsequently aligned to the *H. rubra* reference genome (NCBI RefSeq QXJH00000000.1) (Gan et al. 2019) using the PPalign package in the PoolParty pipeline (Micheletti & Narum 2018) with default parameters. Single nucleotide polymorphisms (SNPs) were called using PoolFstat (Hivert et al. 2018) where SNPs were required to have a read depth 20-200 reads to make a call. SNPs with a minor allele frequency of $\geq 0.05$ were used for downstream genomic analysis.

***Estimating overall genetic structure***

SNP frequencies over all loci were initially contrasted between all 14 sample locations to determine patterns of overall genetic structure and population connectivity. Analyses were repeated following the removal of candidate loci potentially under directional selection (refer to section immediately below) but had little effect. The software PoolFstat implemented in R (Hivert et al. 2018) was used to calculate global and pairwise measures of population differentiation ($F_{ST}$) (Weir & Cockerham 1984).

***Genome-wide association analysis***

To identify SNPs associated with virus exposure status we performed a genome-wide association study (GWAS) using BAYPASS 2.1 (Gautier 2015). Analyses were performed under the auxiliary (AUX) covariate mode (-covmcmc and - auxmode flags), after scaling the variables with the - scalecov flag. The underlying models explicitly account for the covariance structure among the population allele frequencies that originates from the shared history of the populations under study, through the estimation of the population covariance matrix Ω, which removes the variation associated with demography (Bonhomme et al. 2010; Gunther & Coop 2013). The auxiliary covariate model specifically involves the introduction of a binary auxiliary variable to classify each locus as associated or not. This allows the computation of posterior inclusion probabilities (and Bayes Factors) for each locus while explicitly accounting for multiple testing issues. The auxiliary covariate model was applied with default parameters, a 5,000 burn-in of iterations in the Markov chain Monte Carlo (MCMC) chain, followed by 25,000 iterations. To reduce artefacts due to potential variability between runs, we performed 5 independent BAYPASS runs. We then calculated the average Bayes Factor (BF), expressed in deciban units (dB), for each SNP as a quantitative estimate of the strength of association virus exposure and the standardized allele frequency. For each SNP, significance was assessed based on the Bayes Factor (BF) models according to Jeffrey's rule (Jeffreys 1961). Only SNPs with BF scores $\geq 50$, regarded as a decisive association, were retained as potential candidate loci.

***Posthoc analyses including functional annotations.***

We calculated linkage disequilibrium (LD) among candidate loci using LDx, a method which uses an approximate maximum likelihood approach from pooled resequencing data (Feder et al. 2012). Linkage disequilibrium is calculated as $r^2$, that is, the square of the correlation between alleles of SNP pairs within the paired sequence reads of each population. We subsequently calculated the average LD for each pairwise SNP comparison across sample sites. An analysis of principal components (PCA) was implemented in the adegenet package for R (Jombart 2008; Jombart & Ahmed 2011) to obtain a graphical depiction of patterns of genetic structure among virus affected and unaffected stocks based on all candidate SNPs identified by BayPass (BFs > 50).

SnpEff v2.0.3 (Cingolani et al. 2012) was used to map candidate SNP loci to the *H. rubra* annotated genome and to predict variant impacts, estimated as high (highly disruptive impact on protein function), moderate (possible change in protein effectiveness), low (unlikely to change protein behaviour) or a modifier (synonymous coding, non-coding or intergenic variant). Functional classification of candidate genes was achieved by matching the peptide sequences for mapped candidate *H. rubra* genes with the annotated genomes for human (NCBI RefSeq IDs NC_000001 - NC_000024), Pacific Oyster (RefSeq IDs NC_047559 - NC_047568), scallop (RefSeq ID NC_007234.1) and mussel (RefSeq ID NC_006161.1) using the web-based version of the DAVID bioinformatics tool (Huang et al. 2009b; Huang et al. 2009a). Functional annotations were performed by focussing specifically on gene homologs known to be associated with virus-host interactions, in particular herpesvirus response pathways.

# Results

***Genotyping and overall population structure***

Pooled whole genome re-sequencing of 343 *H. rubra* specimens from 14 locations distributed across the Victorian Western and Central Zone Abalone yielded a total of 7,745,655 SNPs that were subsequently used for the downstream analyses. Estimates of overall genetic structure indicated a lack of overall genetic structure and pattern of panmixia across the Western and Central Zone fisheries. Specifically, analyses demonstrated that global $F_{ST}$ did not differ significantly from zero ($F_{ST}$ = 0, $P > 0.05$), nor did any pairwise measure of $F_{ST}$ between sampling locations. These findings are consistent with previous estimates of genetic structure in Victorian *H. rubra* fisheries by Miller *et al.* (2016, 2019).

***Genome-wide association analysis***

Our genome-wide association study found 25,854 SNPs with decisive associations with virus exposure (BF > 50). An inspection of the distribution of alleles at a subset of the loci confirmed a consistent pattern of differences in major allele frequencies between virus affected and unaffected fishing stocks. Estimates of LD were high in all fishing stocks (mean $r^2 = 0.61 \pm 0.01$ *SD*) indicating non-random association of alleles, while comparisons of $r^2$ between virus affected and unaffected stocks did not significantly differ ($P > 0.05$). When plotted across the x- and y-axes, patterns of genetic structuring can be seen differentiating populations varying in historical AVG exposure (Figure 3).

Candidate SNP loci which showed significant associations with virus exposure were successfully mapped to the annotated *H. rubra* genome. SNPeff analyses predicted 333 candidate loci to have moderate effect on protein function (involving nonsynonymous mutations), while 489 candidates were predicted to have low effect, and 24,722 candidates were recognised as non-coding

or intergenic variants. Candidate loci that successfully mapped to *H. rubra* genome peptides sequences, were found to correspond with gene homologs in other animal systems including *Haliotids,* non-Haliotid marine molluscs, crustaceans, and humans. These include 24 gene homologs linked to HaHV-1 immunity in New Zealand pāua (*H. iris*), and 47 genes associated with herpes virus response pathways in Japanese disk Abalone (*H. discus hannai*), Pacific Oyster (*Crassostrea gigas*), decapod crustaceans (*Penaeus monodon* and *Procambarus clarkia*), and humans (Table 2). An additional, 14 peptides mapped to gene homologs associated with virus interactions in various Haliotids (*H. discus hannai*, *H. laevigata*, and *H. ruscefens*), decapod crustaceans (*Penaeus monodon* and *Procambarus clarkia*), and humans (Table 2).

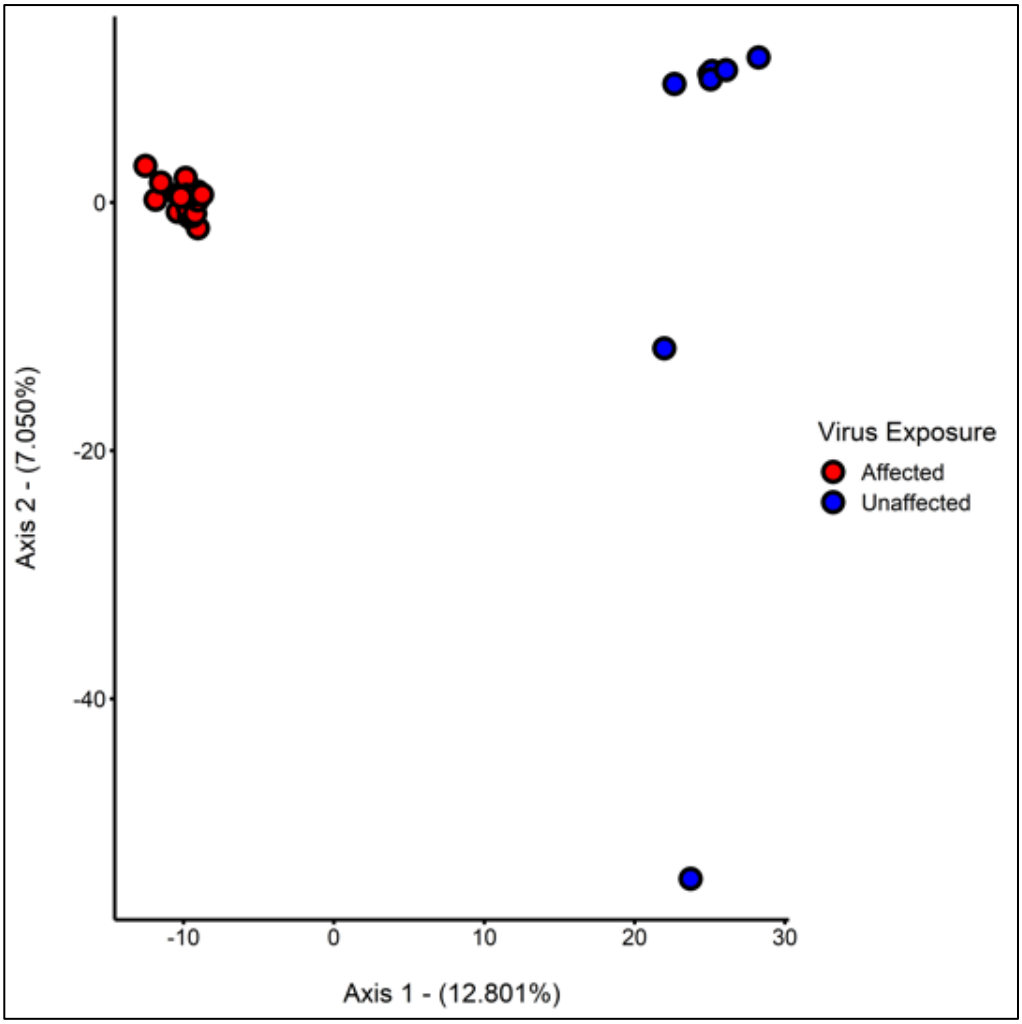


**Figure 3.** Principal Components Analysis plots based on candidate SNPs putatively under selection and associated with historical virus exposure. Each point on the map represents each of the 28 pooled whole genome resequencing libraries for virus affected (red) and unaffected (blue) fishing stocks.

**Table 2.** List of predicted genetic variant impacts, and genes that candidate loci mapped to. Table also includes gene functions, as well as the species from which these functions have been reported and their respective references.

| Predicted variant Impact | Associated gene | Gene function | Species | Reference(s) |
|---|---|---|---|---|
| ***Genes involved H. iris HaHV-1 immune response*** | | | | |
| MODERATE, LOW, MODIFIER | SLC1A2 | Excitatory amino acid transporter 2, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODERATE, LOW, MODIFIER | CYP3A4 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODERATE, MODIFIER | ACE | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | CA2 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | POU6F2 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | NLGN4X | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | CYP3A7 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | Peritrophin 44 like (LOC105317660) | Chitin-binding peritrophin-A domain, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | Uncharacterized LOC105326593 (LOC105326593) | Chitin-binding peritrophin-A domain, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |

| | | | | |
|---|---|---|---|---|
| MODIFIER | CHIT1 | Chitin-binding peritrophin-A domain, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | CYP3A5 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | CYP3A43 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | POU2F1 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | POU3F4 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | Uncharacterized LOC105336951 (LOC105336951) | Chitin-binding peritrophin-A domain, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | Ganglioside GM2 activator like (LOC105346019) | Chitin-binding peritrophin-A domain, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | Ganglioside GM2 activator like (LOC105348613) | Chitin-binding peritrophin-A domain, response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | Zinc metalloproteinase nas 6 like (LOC105319685) | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| MODIFIER | FAT1 | Response to HaHV-1 exposure | *Haliotis iris* | Neave et al. 2019 |
| ***Gene homologs associated with herpesvirus response pathways*** | | | | |
| MODERATE | DDX58 | Herpesvirus response pathway | *Homo sapiens* | Martin et al. 2016 |

| | | | | |
|---|---|---|---|---|
| MODIFIER | RELA | Herpesvirus response pathway, C-type lectin containing or involved in c-type lectin pathway (invertebrate immune response) | *Haliotis discus hannai, Procambarus clarkii, Penaeus monodon* | Nam et al. 2016, Zhang et al. 2018, Qin et al. 2019, Wang & Wang, 2013 |
| MODIFIER | MYD88 | Herpesvirus response pathway, response to OsHV-1 exposure | *Crassostrea gigas* | Tang et al. 2017 |
| MODIFIER | CASP8 | Herpesvirus response pathway | *Haliotis discus hannai* | Nam et al. 2016 |
| MODIFIER | SKP1 | Herpesvirus response pathway | *Homo sapiens* | Yu et al. 2016 |
| MODIFIER | TAF10 | Herpesvirus response pathway | *Homo sapiens* | Wagner & DeLuca, 2013 |
| MODIFIER | ARNTL | Herpesvirus response pathway | *Homo sapiens* | Edgar et al. 2016 |
| MODIFIER | C3 | Herpesvirus response pathway | *Homo sapiens* | Verschoor et al. 2003 |
| MODIFIER | CDK2 | Herpesvirus response pathway | *Homo sapiens* | Schang et al. 2002 |
| MODIFIER | EEF1D | Herpesvirus response pathway | *Homo sapiens* | Boulben et al. 2003 |
| MODIFIER | EIF2AK1 | Herpesvirus response pathway | *Homo sapiens* | Burgess & Mohr 2018 |
| MODIFIER | EIF2S1 | Herpesvirus response pathway | *Homo sapiens* | O'Connel & Liang 2016 |
| MODIFIER | HCFC1 | Herpesvirus response pathway | *Homo sapiens* | Shen et al. 2020 |
| MODIFIER | MCRS1 | Herpesvirus response pathway | *Homo sapiens* | Bader et al. 2001 |

| | | | | |
|---|---|---|---|---|
| MODIFIER | PPP1CB | Herpesvirus response pathway | *Homo sapiens* | Silva et al. 2015 |
| MODIFIER | SRSF7 | Herpesvirus response pathway | *Homo sapiens* | Tang et al. 2019 |
| MODIFIER | Ube2r2 | Herpesvirus response pathway | *Homo sapiens* | Beard et al. 2015 |
| MODIFIER | TRAF6 | Herpesvirus response pathway, C-type lectin containing or involved in c-type lectin pathway | *Haliotis discus hannai, Procambarus clarkii, Penaeus monodon* | Nam et al. 2016, Zhang et al. 2018, Qin et al. 2019, Wang & Wang, 2013 |
| MODIFIER | CREBBP | Herpesvirus response pathway, C-type lectin containing or involved in c-type lectin pathway (invertebrate immune response) | *Haliotis discus hannai, Procambarus clarkii, Penaeus monodon* | Nam et al. 2016, Zhang et al. 2018, Qin et al. 2019, Wang & Wang, 2013 |
| MODIFIER | Myd88 2 | Herpesvirus response pathway | *C. gigas* | Tang et al. 2017 |
| MODIFIER | IKBKB | Herpesvirus response pathway, C-type lectin containing or involved in c-type lectin pathway (invertebrate immune response) | *Haliotis discus hannai, Procambarus clarkii, Penaeus monodon* | Nam et al. 2016, Zhang et al. 2018, Qin et al. 2019, Wang & Wang, 2013 |
| MODIFIER | ALYREF | Herpesvirus response pathway | *Homo sapiens* | Chen et al. 2005 |
| MODIFIER | POLR2A | Herpesvirus response pathway | *Homo sapiens* | Tang et al. 2019 |
| MODIFIER | SRPK1 | Herpesvirus response pathway | *Homo sapiens* | Souki & Sandri-Golden, 2009 |

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| MODIFIER | TAF4 | Herpesvirus response pathway | *Homo sapiens* | Wagner & DeLuca, 2013 |
| MODIFIER | TAF6 | Herpesvirus response pathway | *Homo sapiens* | Wagner & DeLuca, 2013 |
| MODIFIER | TBP | Herpesvirus response pathway | *Homo sapiens* | Zabierowski & DeLuca 2008 |
| MODIFIER | TAB1 | Herpesvirus response pathway | *Homo sapiens* | Jahanban-Esfahlan et al. 2019 |
| MODIFIER | TRAF2 | Herpesvirus response pathway | *Homo sapiens* | Hsu et al. 1997 |
| MODIFIER | Csnk2b | Herpesvirus response pathway | *Homo sapiens* | Carter 2011 |
| MODIFIER | CUL1 | Herpesvirus response pathway | *Homo sapiens* | Amici et al. 2006 |
| MODIFIER | EIF2AK4 | Herpesvirus response pathway | *Homo sapiens* | Burgess & Mohr 2018 |
| MODIFIER | Hnrnpk | Herpesvirus response pathway | *Homo sapiens* | Schmidt et al. 2010 |
| MODIFIER | MED8 | Herpesvirus response pathway | *Homo sapiens* | Wu et al. 2011 |
| MODIFIER | NXF1 | Herpesvirus response pathway | *Homo sapiens* | Johnson & Sandri-Golden, 2009 |
| MODIFIER | PPP1CA | Herpesvirus response pathway | *Homo sapiens* | Silva et al. 2015 |
| MODIFIER | PPP1CC | Herpesvirus response pathway | *Homo sapiens* | Silva et al. 2015 |
| MODIFIER | SRSF1 | Herpesvirus response pathway | *Homo sapiens* | Tang et al. 2019 |

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|---|---|---|---|---|
| MODIFIER | SRSF4 | Herpesvirus response pathway | *Homo sapiens* | Chen et al. 2014 |
| ***Gene homologs associated with host-virus interactions*** | | | | |
| LOW, MODIFIER | HIST1H2AA | Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |
| LOW, MODIFIER | COTL1 | Tropomyosin, Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |
| LOW, MODIFIER | PDIA3 | Protein disulfide isomerase activity, Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |
| LOW, MODIFIER | HSP90AB1 | Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |
| LOW, MODIFIER | Histone H2A (LOC105320412) | Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |
| LOW, MODIFIER | AVIL | Gelsolin domain, Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |
| LOW, MODIFIER | Hsp70 member 4 (HSPA4) | Abalone immune response | *Haliotis laevigata, Haliotis ruscefens, Haliotis discus hannai* | Shiel et al. 2015, Brokordt et al. 2015, Nam et al. 2016 |
| LOW, MODIFIER | QSOX1 | Protein disulfide isomerase activity, Abalone immune response | *Haliotis discus hannai* | Nam et al. 2016 |

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|---|---|---|---|---|
| LOW, MODIFIER | Hsp70 member 12A (HSPA12A) | Abalone immune response | *Haliotis laevigata, Haliotis ruscefens, Haliotis discus hannai* | Shiel et al. 2015, Brokordt et al. 2015, Nam et al. 2016 |
| MODERATE, LOW, MODIFIER | PSMA8 | C-type lectin containing or involved in c-type lectin pathway (invertebrate immune response) | *Haliotis discus hannai, Procambarus clarkii, Penaeus monodon* | Nam et al. 2016, Zhang et al. 2018, Qin et al. 2019, Wang & Wang, 2013 |
| MODERATE, LOW, MODIFIER | PSMD12 | C-type lectin containing or involved in c-type lectin pathway (invertebrate immune response) | *Haliotis discus hannai, Procambarus clarkii, Penaeus monodon* | Nam et al. 2016 |
| MODERATE, LOW, MODIFIER | Hsp70 member 8 (HSPA8) | Abalone immune response | *Haliotis laevigata, Haliotis ruscefens, Haliotis discus hannai* | Shiel et al. 2015, Brokordt et al. 2015, Nam et al. 2016 |
| MODERATE, MODIFIER | TIA1 | Viral translation inhibition | *Homo sapiens* | McCormick & Khaperskyy, 2017 |
| MODERATE, MODIFIER | MAP2K4 | Toll-like receptor activity, innate immunity | *Haliotis discus hannai* | Nam et al. 2016 |

# Discussion

In this study we performed a genome-wide association study to test for signatures of adaptative genetic responses to viral exposure in Victorian *H. rubra* fishing stocks historically impacted by AVG. Our analyses were successful in identifying SNP loci associated with AVG exposure, many of which were mapped to genes known to be involved in virus-host interactions, including herpes virus response pathways, in *Haliotids* and other animal systems. These findings provide the first line of evidence of selection for virus response genotypes in AVG affected *H. rubra* fisheries. While these findings will require experimental validation, particularly if they are going to be linked to resistance (discussed below), they provide insights into genetic changes in AVG affected *H. rubra* fisheries, the resilience of Abalone fishing stocks, and possible opportunities for biosecuring Australia's wild and farmed Abalone fisheries.

Our analyses identified approximately 25,000 SNP loci associated with AVG exposure in *H. rubra*, suggesting that selection has driven advantageous genetic variants to higher frequencies in virus affected populations. While this number of positively associated candidates is a substantial proportion of the total number of SNPs included in our analyses (0.4% of loci), a lot of changes are likely to reflect non-random associations of alleles due to significant linkage disequilibrium. Indeed, patterns of strong linkage disequilibrium (LD) are commonly observed when loci are under strong selection (Hedrick 2009). Functional annotations suggest that 333 candidate loci are likely to have moderate effect on protein function, involving nonsynonymous mutations. While 489 candidates were predicted to have low effect, and 24,722 candidates were recognised as non-coding or intergenic variants, it is possible that some of these loci provide functionally important regulatory roles in genomic processes, such as gene expression, and should not be overlooked.

Functional annotations of candidate loci point to direct associations with genes known to be associated with Abalone herpesvirus-1 (HaHV-1) immunity in the New Zealand pāua (*H. iris*). Recently, Corbeil and Helbig (2019) characterised genes associated with immune response in *H. iris* through gene expression analyses in animals subject to HaHV-1 immersion challenge tests. This study provided the first description of the molecular basis of HaHV-1 immunity in a Haliotid species, findings which are further corroborated by our *H. rubra* genome-wide association study. Specifically, we identified candidate loci associated with virus exposure in *H. rubra* that map to 24 gene homologs contributing to the molecular basis of HaHV-1 resistance in *H. iris*. We also identified additional candidates that mapped to 47 gene homologs associated with herpes virus response pathways in the Japanese Abalone (*H. discus*) and the Pacific Oyster (*Crassostrea gigas*), as well as decapod crustaceans (*Procambarus clarkii, Penaeus monodon*), and humans. A further set of candidate loci mapped to 14 gene homologs associated with human-virus interaction pathways in various Haliotids (*H. discus hannai, H. laevigata, and H. ruscefens*), and other animals systems. These findings strongly support the notion that emergent genomic architectures have resulted in divergent adaptation and possible selection for virus resistance in AVG affected *H. rubra* fisheries.

While our findings are encouraging, quantitative experiments are needed to validate if, and how much, resistance to HaHV-1 is determined by the genotypes identified in our study. The most logical approach will be to perform HaHV-1 exposure challenge tests on animals with the putatively resistant genotypes (Crane et al. 2013; Corbeil et al. 2016). Such investments will require collaborations with industry for collection, genomic screening, and selection of animals for HaHV-1 exposure tests. Assuming these experiments confirm that these genotypes control for some level of virus resistance, further experimental trials will still be needed to test for trait heritability and the potential influence of genotype-environment interactions, to determine if the trait can be controlled in a culture environment (i.e. for breeding purposes). Nevertheless, this study represents the critical step in identifying signature of AVG resistance in Victorian wild *H. rubra* fisheries and genotypes that could be used for management geared toward biosecuring wild and farm fisheries in the future.

The findings from this study have potential implications for the future management of wild and farm Abalone fisheries. Characterising the distribution and prevalence of putative virus resistant genotypes in wild fisheries could help managers to identify stocks expected to be either resilient or vulnerable to AVG re-emergence. Theoretically, strategic stock augmentation activities involving the translocations of animals with AVG resistant genotypes could be used to help increase baseline levels of resistance in individual fishing stocks. However, our results suggest it is likely that there is standing genetic variation for adaptation to virus exposure across the fishery given the lack of overall genetic structure among stocks from Western and Central zone fisheries, demonstrated here and by previous studies (Miller et al. 2016; Miller et al. 2019). These results coupled with the findings of Miller et al. (2019) provide much needed information on the resilience of *H. rubra* populations to changing environmental conditions. Combined, these data sets suggest potential genetic adaptations to temperature, wave energy and current intensity, and now virus exposure. This suggests standing genetic variation may be available for selection to act on to counter future environmental change, assisted by widespread gene flow and a short generation time in this species (~4 years) (Andrews 1999).

Assuming that some resistance is present, there could be opportunities for developing AVG resistant breeding lines to help biosecure farm fisheries from future AVG outbreaks. Specifically, selective breeding from animals with optimal genomic-estimated breeding values (based on resistant genotypes) could help farm fisheries protect local stocks from risks associated with AVG re-emergence (Brokordt et al. 2017). Indeed, such programs have been developed in many farmed mollusc, crustacean and finfish fisheries around the world (Calvo et al. 2003; Kjoglum et al. 2008; Moss et al. 2012; Potts et al. 2021). But as discussed above further work is needed to quantify the degree of resistance determine by the candidate genotypes and the heritability of the trait (Brokordt et al. 2017).

There has been some discussion among industry stakeholders in recent times around the potential for reseeding wild stocks from captive reared animals including AVG resistant animals. While there may indeed be valuable opportunities in this space in the future, much research is needed to assess the risks of rapid selection and domestication of farm reared animals, their survival / fitness in wild settings, and the potential for compromising the genetic integrity of wild fisheries (Araki et al. 2007; Christie et al. 2012; Bolstad et al. 2017). There has also been some discussion around the possibility of achieving AVG resistance in Abalone fisheries through gene editing technologies. For virus resistance traits, gene editing technologies such as the clustered regularly interspaced short palindromic repeats (CRISPR) system, may be applied to identify genetic alterations in related species or populations that have already acquired the necessary resistance adaptation and replicating those changes in target populations (Potts et al. 2021). However, opportunities to engineer new traits through gene editing technologies depends largely on the complexity of the trait. Our results, and those of Corbeil and Helbig (2019), indicate that AVG responses are likely to be a highly complex, polygenic set of traits (potentially influenced by 10s to 100s of genes) and it may be difficult to engineer resistance in Haliotids using current gene editing technologies.

# Conclusion

Our genome-wide association study based on pooled whole genome re-sequencing of 343 *H. rubra* specimens from 14 Victoria fishing stocks varying in historical exposure to AVG indicate patterns of divergent adaptation. Analyses identifying SNP loci associated with AVG exposure, many of which were mapped to genes known to be involved in herpes virus response pathways and general virus-host interactions in Haliotids and other animal systems. Most notably, several loci mapped to genes known to be associated Abalone herpesvirus-1 (HaHV-1) immunity in the New Zealand pāua. These findings provide the first line of evidence of rapid evolutionary changes in AVG affected *H. rubra* fisheries that might reflect selection for virus resistant genotypes and consequently have implications for future management of wild and farm *H. rubra* fisheries. Specifically, the availability of resistant

genotypes may assist in biosecuring wild and farm fisheries in the future through strategic survey, translocation and breeding programs. Despite these encouraging findings, experimental trials will be needed to test the degree of resistance determine by the candidate genotypes (if any) and the heritability of the trait before industry can harness these candidate genotypes for management purposes with confidence.

# Implications

Abalone viral ganglioneuritis (AVG) caused by the Haliotid herpesvirus-1 (HaHV-1) had devastating effects on wild and farmed Abalone stocks in south-western Victorian in the early 2000s. Despite AVG not being recorded in Victoria waters since 2010, it still remains a significant threat to the economic viability of the industry in south-eastern Australia. For the first time we provide evidence of potential AVG resistance in Victorian *H. rubra* fisheries through a genome-wide association study on *H. rubra* stocks varying in historical AVG exposure. We identify a large number of candidate loci that appear to be associated with virus exposure and herpes virus response pathways in other Haliotids, including Abalone herpesvirus-1 (HaHV-1) immunity in the New Zealand pāua. These loci could potentially be used in a number of ways to reduce the vulnerability of wild and farm fisheries to future AVG outbreaks.

1) Characterising the distribution and prevalence of putative virus resistant genotypes in wild fisheries could help managers to identify stocks expected to be either resilient or vulnerable to AVG re-emergence.
2) Strategic stock augmentation activities involving the translocations of animals with AVG resistant genotypes could be used to help increase baseline levels of resistance in individual fishing stocks.
3) The availability of genotypes contributing to AVG resistance could enable the development of genomic selection / selective breeding programs to help biosecure farm fisheries.

These findings provide evidence of rapid evolutionary responses in AVG affected *H. rubra* fisheries and provide valuable insights into the resilience of *H. rubra* populations to changing environmental conditions. Data from this and previous studies (Miller et al. 2019) suggest standing genetic variation may be available for selection to act on to counter future environmental change, assisted by widespread gene flow and a short generation time in this species (~4 years; Andrews, 1999).

However, our findings are based on correlative genomic patterns only and quantitative experiments are now needed to validate if, and how much, resistance to HaHV-1 is determined by the genotypes identified in our study (i.e. HaHV-1 exposure challenge tests). Similarly, experimental trials will be needed to test for trait heritability and the potential influence of genotype-environment interactions, to determine if the trait can be controlled in a culture environment (i.e. for breeding purposes).

# Recommendations

Our original FRDC proposal included a partnership between Deakin University and CSIROs Australian Animal Health Laboratories (now the Australian Centre for Disease Preparedness), and an integrated approach involving both genome-wide association studies and HaHV-1 exposure challenge tests for characterising AVG resistance in *H. rubra* fisheries. This was recognised as a high risk project, and therefore the initial proposal was scaled back to include the genome-wide association as a stand-alone first stage investment. Follow up investment is now needed to finance necessary HaHV-1 exposure challenge tests at ACDP to validate if, and how much, resistance to HaHV-1 is determined by the genotypes identified in our study. This is the critical next step that will enable the industry to

determine if AVG resistance truly persists in *H. rubra* fisheries and can be harnessed for future biosecurity purposes.

# Extension and Adoption

This project was undertaken in direct partnership with wild and farm Abalone fishing industry partners. Milestone reports have been disseminated to project partners throughout the program to provide project updates and details of research progress. Industry partners have also contributed to the drafting of the final report. Due to COVID-19 restrictions our team have not attended industry workshops to present our research findings. However, now that these restrictions have eased, Principal Investigator Adam Miller has been invited to present the findings of this study to the Abalone Council Australia in July 2021 and is also expected to present the research findings at the next Australian Abalone Growers Association annual general meeting (date to be confirmed). As discussed below, no media or additional reporting has emerged from this work to date, and we will await recommendation from the industry partners for us to do so. The findings of this study will be shared with the scientific community via peer-review publication, and we anticipate that the manuscript will be submitted by the end of May 2021.

This project provides the first line of evidence for rapid evolutionary changes and possible virus resistance in AVG affected wild Blacklip Abalone fisheries. Quantitative experiments on animals with candidate genotypes (i.e. virus immersion tests) are now needed to validate if, and how much, resistance is determined by these genotypes. The outcome of these experiments will determine if these genetic markers can be adopted by industry to help biosecure wild and farm fisheries through strategic restocking and breeding programs.

## Project coverage

There hasn't been any media, industry or government articles, or workshops to present findings generated from this project as yet, apart from FRDC milestone reports (which have been circulated to ACA and AAGA industry partners) and a scientific paper currently in draft (see above).

# Appendix one: List of researchers and project staff

| | | |
|---|---|---|
| Principal Investigator | Adam Miller | Deakin University Warrnambool Campus |
| Co-Investigator | Craig Sherman | Deakin University Geelong Waurn Ponds Campus |
| Co-Investigator | Larry Croft | Deakin University Geelong Waurn Ponds Campus |
| Co-Investigator | Ary Hoffmann | University of Melbourne |
| Co-Investigator | Nick Savva | Australian Abalone Growers Association Inc (AAGA) |
| Co-Investigator | Dean M. Lisson | Abalone Council Australia Ltd (ACA) |
| Co-Investigator | Andrew W. Clarke | Victorian Fisheries Authority (VFA) |

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