

# **Antiviral immune responses in abalone and influence of potential abiotic and biotic factors**

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## Contents

|  |    |
|--|----|
| Contents.....  | 2  |
| List of tables .....   | 6  |
| List of figures .....  | 7  |
| Abbreviations .....  | 8  |
| Declaration .....  | 9  |
| Authority of access .....  | 10 |
| Abstract .....   | 11 |
| Acknowledgement.....   | 14 |
| Thesis Structure .....   | 15 |
| CHAPTER 1: General introduction .....  | 17 |
| 1.1. Major economic abalone species in Australia .....   | 17 |
| 1.2. Effects of microbial infection and environmental stress on abalone production .....                           | 19 |
| 1.2.1. Virus infections .....  | 19 |
| 1.2.2. Bacterial infections .....  | 20 |
| 1.2.3. Environmental stress factors .....  | 23 |
| 1.3. Immune responses against microbial infections .....   | 25 |
| 1.3.1. Cell-mediated immunity .....  | 26 |
| 1.3.2. Humoral immunity defense.....   | 27 |
| 1.3.3. Dietary derived anti-microbial factors .....  | 29 |
| 1.4. Research Aims .....   | 30 |
| CHAPTER 2: <i>In vitro</i> antiviral activity against herpes simplex virus in abalone <i>Haliotis laevis</i> ..... | 32 |
| Abstract.....  | 33 |
| 2.1. Introduction.....   | 34 |
| 2.2. Materials and methods .....   | 37 |
| 2.2.1. Abalone .....   | 37 |
| 2.2.2. Cell culture and virus .....  | 37 |
| 2.2.3. Tissue and haemolymph collection.....   | 38 |
| 2.2.4. Lipophilic and non-lipophilic extraction.....   | 38 |
| 2.2.5. Peptide extraction.....   | 39 |
| 2.2.6. Protein determination .....   | 39 |

|  |    |
|--|----|
| 2.2.7. Cytotoxicity assays .....   | 40 |
| 2.2.8. Anti-HSV assay.....   | 40 |
| 2.2.9. Timing of antiviral activity .....  | 41 |
| 2.2.10. Attachment assay .....   | 42 |
| 2.2.11. Entry assay .....  | 43 |
| 2.2.12. Virucidal assay .....  | 43 |
| 2.2.13. Statistical analysis .....   | 44 |
| 2.3. Results.....  | 44 |
| 2.4. Discussion.....   | 53 |
| Acknowledgments .....  | 58 |
| CHAPTER 3: Variation in the antiviral and antibacterial activity of abalone <i>Haliotis laevis</i> , <i>H. rubra</i> and their hybrid in South Australia ..... | 59 |
| Abstract.....  | 60 |
| 3.1. Introduction.....   | 62 |
| 3.2. Materials and methods.....  | 65 |
| 3.2.1. Abalone .....   | 65 |
| 3.2.2. Spawning induction.....   | 66 |
| 3.2.3. Haemolymph collection .....   | 66 |
| 3.2.4. Cell culture and virus .....  | 67 |
| 3.2.5. Anti-HSV assay.....   | 67 |
| 3.2.6. Bacteria .....  | 68 |
| 3.2.7. Antibacterial assay .....   | 68 |
| 3.2.8. Statistical analysis .....  | 69 |
| 3.3. Results.....  | 70 |
| 3.4. Discussion.....   | 76 |
| Acknowledgments .....  | 83 |
| Appendix 3.1. Map of abalone collecting locations .....  | 84 |
| CHAPTER 4: Effects of micro and macroalgal diet supplementations on growth and immunity of greenlip abalone, <i>Haliotis laevis</i> .....                      | 85 |
| Abstract.....  | 86 |
| 4.1. Introduction.....   | 88 |
| 4.2. Materials and methods.....  | 91 |
| 4.2.1. Experimental abalone and diets .....  | 91 |
| 4.2.2. Macro-and microalgae feeding trials .....   | 91 |

|   |     |
|---|-----|
| 4.2.3. Haemolymph analyses .....  | 93  |
| 4.2.4. Lipophilic extraction and antiviral assay .....  | 96  |
| 4.2.5. Statistical analysis .....   | 96  |
| 4.3. Results.....   | 97  |
| 4.3.1. Growth rates .....   | 97  |
| 4.3.2. Haemolymph immune parameters .....   | 99  |
| 4.3.3. Antiviral activity of lipophilic extracts .....  | 103 |
| 4.4. Discussion.....  | 105 |
| Acknowledgments .....   | 110 |
| CHAPTER 5: Influence of elevated temperatures on the immune response of<br>abalone, <i>Haliotis rubra</i> .....                                       | 111 |
| Abstract.....   | 112 |
| 5.1. Introduction.....  | 114 |
| 5.2. Materials and methods .....  | 117 |
| 5.2.1. Field-sampled abalone .....  | 117 |
| 5.2.2. Laboratory temperature challenge .....   | 117 |
| 5.2.3. Haemolymph parameter measurements .....  | 119 |
| 5.2.4. Statistical analysis .....   | 122 |
| 5.3. Results.....   | 123 |
| 5.3.1. Field survey.....  | 123 |
| 5.3.2. Laboratory temperature challenge experiment .....  | 124 |
| 5.4. Discussion.....  | 129 |
| Acknowledgements.....   | 137 |
| Appendix 5.1. Effect of incubation temperature on antibacterial activity.....   | 138 |
| CHAPTER 6: Immunological changes in response to herpesvirus infection in<br>abalone <i>Haliotis laevigata</i> and <i>Haliotis rubra</i> hybrids ..... | 139 |
| Abstract.....   | 140 |
| 6.1. Introduction.....  | 142 |
| 6.2. Materials and methods .....  | 143 |
| 6.2.1. Abalone .....  | 143 |
| 6.2.2. Infection of abalone by immersion .....  | 144 |
| 6.2.3. TaqMan real-time PCR .....   | 146 |
| 6.2.4. Haemolymph analyses .....  | 147 |
| 6.2.5. Statistical analysis .....   | 148 |

|   |     |
|---|-----|
| 6.3. Results.....   | 149 |
| 6.3.1. Viral infection and TaqMan real-time PCR.....  | 149 |
| 6.3.2. Immune responses at subclinical stage of viral infection .....   | 151 |
| 6.3.3. Immune responses at clinical stage of viral infection by immersion (days<br>5, 6, 7 post-challenge)..... | 153 |
| 6.4. Discussion.....  | 155 |
| Acknowledgements.....   | 160 |
| Appendix 6.1. Infection of abalone by injection .....   | 161 |
| Methods.....  | 161 |
| Results .....   | 162 |
| Appendix 6.2. Standard curve between plasmid Topo-ORF49 and C <sub>T</sub> value .....                          | 164 |
| CHAPTER 7: General discussion .....   | 165 |
| References .....  | 173 |

**List of tables**

|           |   |
|-----------|---|
| Table 1.1 | The reported infectious bacteria and viruses in abalone   |
| Table 1.2 | Proposed mode of actions for antiviral compounds  |
| Table 2.1 | Cytotoxicity and antiviral activity of haemolymph and lipophilic, non-lipophilic, and peptide extracts of different tissues from abalone                            |
| Table 2.2 | Antiviral activity of acyclovir, abalone haemolymph and lipophilic extract of digestive gland   |
| Table 2.3 | Characterisation of antiviral activity in abalone haemolymph  |
| Table 2.4 | Characterisation of lipophilic or non-lipophilic component in abalone haemolymph for antiviral activity   |
| Table 4.1 | The impact of macro- and microalgae supplementations on shell gain and body weight gain of greenlip abalone   |
| Table 4.2 | The effect of diet and feeding period (fixed factors) on the overall immune status (multivariate PERMANOVA) and specific immune parameters (univariate PERMANOVA)   |
| Table 4.3 | Effect of different diets on percent antiviral activity of lipophilic extract of abalone digestive gland against HSV-1  |
| Table 5.1 | The effect of temperature and length of exposure (fixed factors) on individual immune parameter (univariate PERMANOVA) or combined four different immune parameters |

## List of figures

|          |   |
|----------|---|
| Fig. 1.1 | The "holy triad" of disease causality: interactions between the molluscan host, environmental factors and pathogens   |
| Fig. 2.1 | Differing infection protocols for addition and residency times of abalone extracts relative to virus-cell incubation  |
| Fig. 2.2 | Activity against HSV-1, as % reduction in plaque numbers, for abalone haemolymph, lipophilic extract of digestive gland and acyclovir   |
| Fig. 2.3 | Antiviral activity of abalone A) haemolymph and B) lipophilic extract of digestive gland  |
| Fig. 3.1 | Variation in A) antiviral activity (%) and B) antibacterial activity (%) at individual, family line, population and species scale   |
| Fig. 3.2 | Comparison of A) antiviral activity (%) and B) antibacterial activity (%) between farmed and wild abalone   |
| Fig. 3.3 | Correlation between the shell length (cm) and A) antiviral activity (%) and B) antibacterial activity (%)   |
| Fig. 3.4 | Comparison of A) antiviral activity (%) and B) antibacterial activity (%) between pre- and post-spawning  |
| Fig. 4.1 | Summary of macroalgae and microalgae feeding experiments  |
| Fig. 4.2 | Effect of macroalgal diets on immune status of <i>H. laevisgata</i>   |
| Fig. 4.3 | Effect of microalgae supplementations on immune status of <i>H. laevisgata</i>  |
| Fig. 5.1 | The "holy triad" of disease causality: interactions between molluscan host, environmental factors and pathogens   |
| Fig. 5.2 | Summary of laboratory temperature challenge experiment  |
| Fig. 5.3 | The relationship between antiviral and antibacterial activity and water temperature   |
| Fig. 5.4 | Effect of challenge temperature and length of exposure on (a) total haemocyte count (THC, cells x 10 <sup>4</sup> per ml), (b) superoxide anion (SO, OD 630nm), (c) antiviral activity (%) against HSV-1 and (d) antibacterial activity (%) against <i>Vibrio anguillarum</i> |
| Fig. 5.5 | Principal coordinates plot showing the grouping of abalone according to temperature   |
| Fig. 6.1 | Summary of the abalone herpesvirus challenge trial  |
| Fig. 6.2 | Quantification of AbHV gene copies (log <sub>10</sub> ) based on ORF49 qPCR Ct values, in apparently healthy and moribund abalone from day 5 post-infection   |
| Fig. 6.3 | Effect of AbHV infection and time post-infection on A) total haemocyte count (THC, cells x 10 <sup>4</sup> per ml), B) superoxide anion (SO, OD 620nm), C) antiviral activity (%) against HSV-1   |
| Fig. 6.4 | Effect of AbHV infection status at the clinical stage on abalone immune responses   |

**Abbreviations**

|       |  |
|-------|--|
| AbHV  | Abalone herpesvirus                              |
| AVG   | Abalone viral ganglioneuritis                    |
| DMSO  | Dimethyl sulfoxide                               |
| EMEM  | Eagle's minimal essential medium                 |
| HSV-1 | Herpes simplex virus type 1                      |
| NBT   | Nitroblue tetrazolium                            |
| OD    | Optical density                                  |
| PBS   | Phosphate buffered saline                        |
| PCR   | Polymerase chain reaction                        |
| PFU   | Plaque-forming unit                              |
| qPCR  | Quantitative real time polymerase chain reaction |
| RT    | Room temperature                                 |
| SO    | Superoxide anion                                 |
| THC   | Total haemocyte count                            |



**Declaration**

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.



12/11/2012

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Vinh Dang

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## Abstract

Abalone (Haliotidae) are marine gastropod mollusks and important aquaculture species worldwide. Unfortunately, severe mortality of abalone caused by a herpesvirus (AbHV) has been reported in Australia. The manifestation of disease involves an interaction between virus, environment and abalone immunity (Chapter 1). Therefore, this research aimed to investigate the presence of antiviral activity in abalone *Haliotis laevigata* (greenlip), *H. rubra* (blacklip) and their hybrid. Due to the lack of molluscan cell lines for culturing AbHV, antiviral activity of abalone was assessed against a similar neurotropic herpesvirus, herpes simplex virus type 1 (HSV-1) using the plaque assay. Assessment of antiviral activity was complemented with other immune assays, measuring total haemocyte count (THC), phagocytosis, phenoloxidase activity, respiratory burst and antibacterial activity against *Vibrio* spp. to provide an overall view of immune status in abalone after exposure to various biotic and environmental factors.

A number of abalone organs were screened for anti-HSV-1 activity, but only the haemolymph (20%, v/v) and the lipophilic extract of digestive gland (3,000  $\mu\text{g ml}^{-1}$ ) were found to substantially decrease the number and size of virus plaques (Chapter 2). Haemolymph inhibits viral infection at an early stage (e.g. viral entry) whereas the antiviral effect of the lipophilic extract is greatest when added one hour after infection (e.g. the intracellular stage of viral infection).

There was considerable variation in the levels of antiviral and antibacterial activity in the haemolymph among abalone within the same aquaculture family lines and natural populations in different geographic locations (Chapter 3). Antiviral and antibacterial

activity increased slightly with an increase in shell length. However, there was no significant effect of gender or spawning status on antiviral or antibacterial status.

Concomitant with strong antiviral activity against HSV-1 in a lipophilic extract of *Ulva lactuca* and *Spyridia filamentosa*, higher antiviral activity was detected in the digestive gland lipid extract of abalone fed *Ulva lactuca* (64.2% at 650 $\mu$ g ml<sup>-1</sup>) or *Spyridia filamentosa* (69.51%) compared to abalone fed pellets (47.42%) or pellets supplemented with *Arthrospira maxima* (46.3%) or *Dunaliella salina* (46%) (Chapter 4). There was no influence of diet on the humoral antiviral activity, indicating antiviral factors in the haemolymph are likely to be innately biosynthesized by the abalone.

Sampling of wild-caught *H. rubra* showed a significant correlation between temperature and antiviral or antibacterial activity, with higher activity in summer than in winter months (Chapter 5). However, antibacterial activity was compromised in favour of antiviral activity as the water temperatures peaked in summer. A controlled laboratory experiment with water temperature raised from 18 to 21 or 24 °C showed that THC and SO increased at day 1 and then dropped back to control levels by days 3 and 7. By comparison, the humoral immune parameters showed a delayed response with antibacterial and antiviral activity significantly increasing on days 3 and 7, respectively. Consistent with the field study, antibacterial activity became significantly depressed after prolonged exposure to elevated temperatures. Consequently, abalone may have more resilience to viruses than bacterial pathogens under conditions of elevated temperature.

Experimental transmission trials were undertaken using an immersion model to study the abalone immune response to infection with AbHV (Chapter 6). The infection

status of abalone was confirmed by real-time PCR. THC decreased by 38.8% in moribund abalone, but increased by 42.6 and 13.6% in apparently healthy abalone that were PCR-negative and PCR-positive for AbHV, respectively, in comparison to the non-infected group. The level of SO decreased in abalone confirmed as PCR-positive for AbHV, by 30.8% in moribund abalone and by 7.2% in apparently healthy abalone. However, for apparently healthy abalone that were PCR-negative after viral challenge, SO significantly increased, by 59.3%, in comparison to uninfected controls. These results suggest that THC and SO provide potential immune markers for AbHV infection status.

In conclusion, abalone have at least two antiviral compounds with different modes of action against viral infection. Humoral antiviral factors appear to be constitutively produced and are influenced by high temperature but not by diet or infection status. Further investigation is required to establish whether the individual variability in antimicrobial activity is heritable in breeding programs and whether higher activity confers greater resistance to disease.

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## **Thesis Structure**

This thesis encompasses five discrete experiments, each of which is presented in an independent manuscript format. Thus, some repetition of background and methods may be found between chapters. Each chapter has independent hypotheses and aims, and these complement the overall aims mentioned in the introductory chapter. For chapters that have been published or have been accepted for publication in peer-reviewed journals, the literature citation is given on the title page of that chapter. Within each manuscript, the “study” refers to the particular experiment associated with only that chapter. References to other experiments are made by formal citation of published or submitted work. Although the nominative form “we” is used in journal submitted manuscripts of Chapter 2-6, the work presented was undertaken by myself under supervision of the coauthors.

My supervisors, Peter Speck and Kirsten Benkendorff, are listed on all papers due to intellectual input in experimental design, interpretation and feedback on manuscript drafts. Contributions of other co-authors are listed for chapters below.

- Chapter 3: Prof. Mehdi Doroudi is a named investigator on the original Seafood CRC grant that funded this project and he had intellectual input into the idea of screening different geographic populations and family lines. Ben Smith is my industry mentor and he facilitated access to farmed family lines and helped set up the spawning experiment.
- Chapter 4: Yan Li facilitated bacterial culture for antibacterial assay and helped set up the phagocytosis assay.

- Chapter 6: Dr Mark Crane is a co-investigator on the Seafood CRC grant that funded this research and he facilitated the experiments at AAHL, arranged animal ethics approval for the infection experiment and had intellectual input into the experimental design and interpretation. Dr Serge Corbeil and Lynette Williams helped set up the infection experiments and facilitated the PCR and interpretation. John Hoad facilitated cell culture work at AAHL.

Chapter 1 is a general introduction to the concepts involved in this research, as well as a presentation of the aims and significance of this work. Chapter 2 contains a manuscript, published in *Journal of General Virology*, that describes the *in vitro* antiviral activity against herpes simplex virus in abalone *Haliotis laevis*. Chapter 3 contains a paper published in *Aquaculture* that describes variation in the antiviral and antibacterial activity of abalone *H. laevis*, *H. rubra* and their hybrid in South Australia. Chapter 4 contains a manuscript, published in *Aquaculture*, which identified the effects of micro- and macro-algal diet supplementations on growth and immunity of greenlip abalone, *H. laevis*. Chapter 5 contains a manuscript published in *Fish and Shellfish Immunology* that documents the influence of elevated temperatures on immune responses of abalone, *H. rubra*. Chapter 6 contains a manuscript, submitted to *Fish and Shellfish Immunology* (3<sup>rd</sup> July 2012), which describes immunological changes in response to herpesvirus infection in abalone *Haliotis laevis* and *Haliotis rubra* hybrids. Chapter 7 represents a general conclusion and summary of the entire study, drawing together the results and implication from all papers.